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# SOIL SCIENCE

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FELIX LÖHNIS

# Felix Löhnis

## 1874–1930

Doctor Felix Löhnis, world famous agricultural bacteriologist, died in Leipzig, Germany, on December 8, 1930. Doctor Löhnis was born in Dresden, Germany, on August 3, 1874, and obtained his academic education in the Universities of Jena, Halle, and Leipzig, obtaining his PhD. from the latter in 1901. After teaching for two years in agricultural schools, he returned to Leipzig and in 1905 was made head of agricultural bacteriology, which position he held until 1914. In this capacity, he was responsible for instruction and research, resulting in the publication of numerous papers on milk and soil bacteriology. A manual of methods, *Landwirtschaftlich-bakteriologisches Praktikum*, made its appearance during this period and was subsequently translated into English, French, Japanese, Polish, and Russian. In 1910 he published the well-known *Handbuch der landwirtschaftlichen Bakteriologie*, the only comprehensive review of the literature in existence at that time. These and two other books, *Einführung in die Bakteriologie* and *Vorlesungen über landwirtschaftliche Bakteriologie*, gained an international reputation for him.

In 1914, a few months before the outbreak of the World War, Dr. Löhnis accepted an appointment in the Office of Soil Bacteriology, U. S. Department of Agriculture, becoming the chief of that office in 1923. Soon after taking up his research in America, he investigated the morphologic changes in cultures of Azotobacter and developed the theory of a definite life cycle, later extending this work to include other bacteria. A review of the literature up to 1918, published as a memoir of the National Academy of Sciences, was done in characteristic Löhnis fashion. Whether one agrees with his views on life cycles or not, it is certain that his work on this subject has exerted a profound influence upon the science of bacteriology, the development of which only the future can tell.

Besides the life cycle work, he carried on field and greenhouse experiments for 10 years on the effect of legumes on succeeding crops, the nitrogen availability of green manures, and allied subjects. During this period, a *Textbook of Agricultural Bacteriology* (with E. B. Fred) made its appearance.

In 1925, Dr. Löhnis resigned as chief of the Office of Soil Bacteriology, the position he had held for two years, and returned to the University of Leipzig as professor and director of the Institute of Agricultural Bacteriology and Soil Science. Students again flocked to his laboratory and in a relatively short time a great amount of research work was undertaken. In addition to the work incident to his teaching and lectures before agricultural groups, he issued a second edition of his *Vorlesungen* in 1926, and in 1929 became editor of the

*Centralblatt für Bakteriologie* Abt. II. The second edition of the voluminous *Handbuch* was in the course of preparation at the time of his death.

Relatively few Americans knew Löhnis personally. His partial deafness made it unpleasant for him to attend meetings, which he rarely did. His great knowledge of the literature and his ability to recall it made him at once a helpful friend or a severe critic. He was very intolerant of work poorly done, of old facts published as new, or of over-emphasis as to the importance of the work, and he did not hesitate to express himself and in very choice language. Consequently many who came in contact with him only through publication or correspondence knew only that side of his nature; but those who had the privilege of working with him, will long remember a very different personality, jovial, enthusiastic, and always ready to help any investigator who wished to work and showed that he was sincere. Untiring and thorough in his work, whether searching through the literature or looking through a microscope, he was an inspiration to those under his direction; the pity of it is that more persons could not have profited by such an association. His death came as a distinct blow to his former associates, and will mean a great loss to the science of agricultural bacteriology throughout the world.

NATHAN R. SMITH.

# CONSERVATION AND AVAILABILITY OF THE NITROGEN IN FARM MANURE

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The beneficial effect of animal manure when applied to farm crops has been recognized from very early times. More recent information leads us to believe that this beneficial effect is due, in the main, to the three elements, phosphorus, potassium, and nitrogen, and more especially to the nitrogen, which the manure contains. The value of farm manure has been placed at figures ranging from $2 to $5 a ton, depending on the kind and quality of the manure and the type of farming practice. At these figures, the average dairy cow will produce during the year manure valued at from $26 to $65. Farm manure perhaps has its greatest economic value in the more intensive agricultural districts and to a lesser extent in the districts where dairy farming or general livestock farming is practiced.

During the year, the average dairy cow produces about 13 tons of manure containing 130 pounds of nitrogen, of which about 70 pounds are in the liquid portion. Most of the present investigations indicate that the nitrogen in the liquid manure has the same value as the nitrogen in sulfate of ammonia. At this rate the nitrogen in the liquid manure alone from one cow for one year has a value of about $15. There is grave doubt whether more than half of this nitrogen is utilized on the American dairy farm; in fact there are data to indicate that as much as half of it may be lost. If this is true, the dairy farmers of the state of Wisconsin alone are losing annually over $20,000,000 worth of readily available nitrogen.

Because of the value of the nitrogen in farm manure and the ease with which it is lost, much work has been done on its composition, conservation, and utilization, especially by European workers. Some of this work will be reviewed briefly in relation to the work reported in this paper.

*Composition.* The nitrogen content of cattle manure varies with several factors, including the kind of animal, kind of feed, season of the year, amount and kind of bedding, and methods of handling the manure. The excrement from cattle is made up of about 70 per cent solid and 30 per cent liquid by weight. The nitrogen content of the dung will vary from 0.20 to 0.45 per cent with an average between 0.35 and 0.40 per cent and the urine from 0.80 or less to over 1.5 per cent. If the complete excrement from the cow is mixed with

[1] From the department of soils. Published with the approval of the director.

enough straw as bedding to absorb the liquid, a complete manure is obtained which contains from 0.50 to 0.55 per cent of nitrogen, half of which is from the liquid manure. If this manure is allowed to stand for a few days the nitrogen from the liquid manure changes to ammonia so that the result is a manure containing 10 to 12 pounds of nitrogen a ton, of which 5 to 6 pounds are in the form of ammonia.

Hansen (15) reports the nitrogen content of stable manure as ranging between 0.5 and 0.6 per cent where the total excrement is included. Of the total nitrogen from 25 to 40 per cent is in the ammonia form. Analyses of manures from the farms in Jutland and Seeland, Denmark (27) show from 0.43 to 0.56 per cent of nitrogen. The Ohio station (2) gives figures to show that the manure, including the litter, from dairy cattle contains 0.57 per cent nitrogen of which 0.285 per cent is water soluble. Analyses of manure from the Hancock (Wisconsin) substation show 0.66 per cent total and 0.18 per cent ammonia nitrogen, and samples from the Marshfield (Wisconsin) substation a total nitrogen content of 0.56 per cent of which not more than 20 per cent is ammonia nitrogen. These figures show that there is less ammonia nitrogen in the samples taken from the farms than should be found if the total excrement is composted with the bedding. This shortage indicates that either there has been a loss of ammonia nitrogen from the manure heap or part of the liquid manure nitrogen is not incorporated with it.

A method, used extensively in Europe, of handling the liquid manure is the use of the manure cisterns. The liquid is separated, as far as possible, from the solid and is stored in underground cisterns. Danish workers (19, 25, 26, 27) have shown that the nitrogen content of the liquid manure from these cisterns varies greatly. On farms where average feeds are used the liquid ranges from 0.45 to 0.55 per cent of nitrogen. If the cisterns are closed tightly there is more nitrogen than where they are more loosely closed or partly open. The nitrogen content varies greatly from the top to the bottom of the tank, ranging from 0.2 per cent at the top to over 0.7 per cent at the bottom. With the exception of from 5 to 10 per cent, this nitrogen is all in the form of ammonia. These workers found that the decrease in nitrogen content from the bottom to the top of the liquid manure is most marked in the surface 2 feet and is due to the loss of ammonia nitrogen by volatilization. This loss is greatest in the poorly covered cisterns. They found that there is no difference in the nitrogen content of the liquid on the same level, whether near the center or near the wall of the cistern. Swiss workers (31) found the nitrogen content of liquid manure in cisterns to range from 0.03 to 0.58 per cent. Five years' analyses of liquid manure gave the average figures of 0.34 per cent total nitrogen and 0.31 per cent ammonia nitrogen. When these data are compared with the fresh urine from cattle containing from 1.0 to 1.3 per cent of nitrogen it seems that there must be a very heavy loss of nitrogen between the animal and the field.

In any manure where the liquid portion is present, the first action to take

place is the change of urea nitrogen to ammonia nitrogen and this change is accompanied by a rise in the pH value. Under normal temperature conditions, this change takes place within about a week. After this there is an acid fermentation similar to that occurring in silage. This fermentation is anaerobic and takes place during a period of from one to two months. During this fermentation carbon dioxide and the volatile organic acids are formed, which in all probability consist largely of acetic and butyric acids. During this fermentation the pH value moves down in proportion to the ratio of fermentable carbonaceous material to ammonia present. Hansen (15, 16) has shown that the pH value of the fermented mixture ranges from 7.0 to 9.0 and the ratio of volatile acids to ammonia varies from 0.85 to 2.53. These biochemical changes leave the liquid manure nitrogen largely in the form of ammonium salts of carbonic and volatile organic acids.

*Conservation of manure nitrogen.* Aside from mechanical losses, the losses of nitrogen from farm manure come for the most part either in its storage until it is hauled to the field or during the hauling and spreading process and until it is incorporated with the soil.

When the liquid, solid, and bedding are stored together the least loss of nitrogen occurs when the manure is tightly packed and contains all of the water it will hold without drainage. Under these conditions, which are practically anaerobic, manure may be held in containers or manure pits with open tops for long periods with very little loss of nitrogen. Russell and Richards (36) think that this may best be accomplished by use of the "deep stall" and the manure allowed to remain under the animal until it is used. This procedure is not impossible in the dairy industry but makes sanitation more difficult. In some districts a large "deep stall" feeding shed is used where the cows run loose and are placed in the stanchions only for milking. It is doubtful whether this scheme can ever be a practical working plan because of the nature of cattle manure. These English workers report that under anaerobic conditions there is very little loss of nitrogen, but that if the manure is mixed and left somewhat aerobic as much as one-third of the nitrogen may be lost. They seem to think that some of it may be lost as elemental nitrogen. Köhnlein (23) quotes figures to show that only 13 per cent of the nitrogen is lost in 4½ months from the deep stall and that if the manure is removed and piled in a more or less open heap as much as 36 per cent is lost when the pile is covered and 37 per cent when not covered. Danish workers (17, 28) have shown that where both the liquid and solid are stored together with the bedding in bins or storage houses with tight bottoms and the manure is packed solid and wet, the loss of nitrogen may be as low as 2 to 5 per cent of the total. If the conditions are less favorable the loss is greater. Work done at this laboratory with large and small containers indicates that where moist anaerobic conditions are maintained, the loss of nitrogen is very small, and occurs for the most part during the period of ammonification which comes during the first week of storage. After this period, fermentation takes place and the ammonia is held in solution as the salts of volatile acids which are rather stable except on drying.

*Storage of liquid.* Where the liquid manure is stored separately from the solid it is best done in cisterns or closed containers. European farmers (27, 31) have followed this practice for many years. The cisterns are usually underground and the liquid is either pumped out or the cistern is built on a hillside and emptied by gravity directly into the spreading tank. These reservoirs range from 12 to 20 feet in diameter and from 8 to 10 feet in depth and with a capacity of from 25 to 60 cubic feet for each cow, the size depending on the size of the herd and the length of the storage period. These cisterns are usually built with walls of concrete or masonry and plastered on the inside. The tops are closed and made as nearly air tight as possible.

Since the nitrogen in stored liquid manure is largely in the form of ammonium carbonate it is easily lost. Jensen (21, 22) has shown that there is a vapor pressure to an ammonia solution directly proportional to the alkalinity. He has also shown that this is likewise true of the ammonium salts of the volatile acids. These facts help to explain the reports of Kristensen (25, 26) that the nitrogen in the liquid manure decreases from the bottom toward the top of the liquid, and that the top 2 or 3 feet may contain as little as 0.2 per cent of nitrogen, because of the volatilization of the nitrogen as ammonia from the liquid. If the ammonia can be changed to a salt of one of the more stable acids, such as the nitrate, sulfate, chloride, or phosphate, then there is no vapor pressure of ammonia and hence it is not lost.

To prevent the loss of nitrogen from the liquid manure, different means have been tried with varied success. Attempts have been made to use disinfectants (11, 12, 35) in order to slow up the ammonification process and thus slow up the losses of ammonia nitrogen. Success from this measure has been small. Kainite and gypsum have also been used in this rôle with only meager success. Ammonification may be slowed up by low temperature (9, 12) but the control of temperature is impracticable. Chemical treatment (12, 22, 23, 29) offers a means to prevent volatilization of ammonia nitrogen by the use of such substances as calcium sulfate, calcium chloride, or calcium nitrate, when the calcium is precipitated as chalk and the ammonia is held as the less volatile sulfate, chloride, or nitrate. Soluble phosphates such as superphosphate or treble superphosphate may also be used in the same way. One of the strong mineral acids (10, 11), such as sulfuric or phosphoric will also hold the nitrogen in a less volatile form. Although these treatments are more or less effective, either their cost of some other factor makes them impracticable. Danish workers (19, 27) found that the more tightly the cisterns are closed the less is the loss of ammonia nitrogen by volatilization and that the cistern must be sealed air tight to prevent the loss entirely. A layer of oil (8, 9, 35) over the surface of the liquid manure has been found to reduce materially the losses from volatilization. Blanck (9) also found that dilution of the liquid manure reduced the loss of ammonia nitrogen. This is no doubt due to the reduction of vapor pressure of the ammonium carbonate.

*The losses of nitrogen from handling manure.* If fresh manure is hauled

directly to the field from the animal before any ammonification of the urea takes place there is less loss of nitrogen because neither the urea or dung nitrogen is volatilized before ammonification. If, on the other hand, the manure has gone through the ammonification period or has passed on through the fermentation process, the urea or liquid manure nitrogen has changed to the ammonium salts of carbonic or volatile organic acids and will be lost by volatilization when the damp manure is exposed to drying. Hansen (15, 16) and Iversen (20) report that when manure is spread out on zinc plates it loses water, ammonia nitrogen, and volatile acids. At 25°C. it loses 75 per cent of its weight and 75 per cent of its ammonia nitrogen in four days. In the open the loss is greatest in clear, warm spring weather with wind, and this amounts to 25 per cent of the total nitrogen in four days. Availability experiments conducted at the Danish stations indicate that a loss of one-half of the ammonia nitrogen may occur from complete fermented manure on being spread and exposed during favorable drying weather for 24 hours before its incorporation with the soil.

There are also losses from handling the liquid manure. Liechti and Ritter (30), found that when the liquid is spread on the soil by sprinkling there is a loss of from 4 or 5 per cent to as much as 20 per cent of the total nitrogen. They found little advantage where superphosphate is used. Houcamp and Blanck (18) found that, when incorporated with the soil, urine and liquid manure give about the same availability of nitrogen as calcium nitrate, sodium nitrate, and ammonium sulfate, but when top-dressed, the returns are less. If the urine or liquid manure is treated with sulfuric or phosphoric acid it gives the same yield whether top-dressed or plowed under. Blanck (9) found that the dilution of liquid manure materially reduces the losses of ammonia nitrogen by volatilization. He (10) also showed that when the untreated liquid manure is applied to the surface of a sandy soil there are heavy losses of nitrogen. The higher the temperature and the older the liquid manure, the greater are these losses. Less of the nitrogen is lost from the liquid manure when applied beneath the surface or mixed immediately with the soil. Machines (24, 38) for this purpose are used by European farmers with cultivated crops in the intensive farming districts but are not so practical on the extensive American farm.

Liquid manure as such, is little used on American farms, generally on account of their extensive nature. Teutsch (39), however, reports the use of liquid manure on pasture lands in the dairy district of western Oregon.

*Availability of manure nitrogen.* Figures showing the availability of the nitrogen in farm manure vary greatly, depending much upon several factors such as quality and relation of soluble to insoluble nitrogen, method of handling, amounts of manure applied, and the crop grown. Hansen (17) reports that where field applications of farm manure are made at the rate of 9,000 kgm. per hectare (about 4 tons per acre) from 37.6 to 43.6 per cent of the nitrogen is available. If one-half this quantity is applied, the availability is from 48.4 to

57.8 per cent, but if one and one-half times this quantity is used, the availability drops to 33 per cent of the total nitrogen applied. At the same time he found that mineral nitrogen gives an availability of 56.0 to 64.5 per cent of the nitrogen applied. Iversen (20), reporting results from two of the Danish stations, shows that at Lyngby for the years 1910–1921, it required 2.33 units of manure nitrogen to equal 1 unit of inorganic nitrogen and at Askov the requirement was 1.9 units of manure nitrogen to equal 1 unit of inorganic nitrogen. These figures indicate that over half of the nitrogen in good farm manure is not available for crop growth. This is borne out by the work of Barthel and Bengtsson (3, 6, 7) in which they show that it is only the ammonia nitrogen, in the main from the liquid manure, that is available to the growing crop. These workers maintain that it is by the conservation and use of the liquid manure that the greatest returns are obtained and that the value of the manure depends very much on this factor. The means of storage and the fermentation through which it goes determine to some extent the value of the manure and the availability of its nitrogen. Scheibe (37) found that manure stored and fermented in closed containers suffers less loss, decomposes more readily in the soil, and the nitrogen is better utilized by crops. Goeters (13) reports that a 3 month's fermentation increases the availability of the nitrogen from 6.6 per cent to 18.4 per cent.

The availability of the nitrogen in farm manure at English and American experiment stations varies greatly and falls generally below that reported by Danish workers. Hall (14) reports the recovery of only 30 per cent of manure nitrogen by mangels, whereas from mineral nitrogen and rape cake the recovery was from 60 to 70 per cent. There was an accumulation of nitrogen in the plot where manure was applied. Apparently the effect of this increase in soil nitrogen is seen in the barley yields on Hoose field for a period of 40 years after the application of manure had been discontinued.

From the New Jersey station Lipman and Blair (32) report 20 years' work on the availability of nitrogen in farm manure and other fertilizers. This work was done in tanks under controlled conditions and showed only 32.7 per cent of the nitrogen in manure available as against 62.4 per cent from sodium nitrate. Later work from this station (33) reports field work showing the following availability of cow and horse manure compared with sodium nitrate for four consecutive 5-year periods:

| | PER CENT OF AVAILABILITY | | | |
|---|---|---|---|---|
| | First 5 years | Second 5 years | Third 5 years | Fourth 5 years |
| 16 T. cow manure—no lime | 14.9 | 14.6 | 18.7 | 21.2 |
| 16 T. horse manure—no lime | 14.6 | 16.2 | 24.6 | .... |
| 160 lbs. $NaNO_3$—no lime | 23.5 | 16.3 | 16.3 | 25.1 |
| 16 T. cow manure—lime | 13.6 | 12.8 | 13.2 | 9.9 |
| 16 T. horse manure—lime | 11.9 | 10.9 | 14.8 | .... |
| 160 lbs. $NaNO_3$—lime | 37.6 | 39.1 | 15.7 | 32.7 |

Analyses of the soil at the end of the 20-year period show that a large part of the nitrogen not recovered from the farm manure is found accumulated in the soil, but there is no accumulation of nitrogen in the plots treated with sodium nitrate. The effect of lime on the availability of manure nitrogen at New Jersey is similar to the findings of Barthel and Bengtsson (4, 5) who report no beneficial effects from either quicklime or limestone, and that large amounts of lime are inhibitory.

The returns from the use of manure nitrogen at the Ohio Agricultural Experiment Station (34) are rather indefinite but interesting nevertheless. By applying to the crop yields average figures for their nitrogen contents, the amount of nitrogen recovered in the crop is obtained, and by comparing the manured plots with the plots receiving approximately the same amount of phosphorus and potassium contained in the manure, an approximate availability per cent for the manure nitrogen is obtained. During a 15-year period on a 5-year rotation of corn, oats, wheat, and two years of hay at Strongville, 8 tons of farm manure shows no recovery of nitrogen from the manure, either on the limed or float treated land. At Wooster on a 5-year rotation of corn, oats, wheat, clover, and timothy, 8 tons of manure applied once in the rotation shows no recovery of the manure nitrogen during a 29-year period. Double this amount of manure gives a 33 per cent recovery of the nitrogen. On a 4-year rotation of corn, oats, wheat, and clover at Wooster, 4 tons of manure an acre for each rotation gives no recovery, but 8 tons an acre shows a recovery of 10 to 12 per cent of its nitrogen over a period of 34 years. From the very nature of the methods used to obtain these figures, it can be easily seen that they are not absolute; in fact they are somewhat contrary to ordinary experience and also to the findings of Danish workers, who report the greatest percentage returns from the lowest per acre application of manure. Although the significance of these figures is doubtful, when taken together with other availability figures there is an indication that the availability of the nitrogen in American farm manure is much lower than is generally thought, no doubt because of the poor storage and handling methods and also its low ammonia content.

### GREENHOUSE WORK ON THE AVAILABILITY OF MANURE NITROGEN

Two sets of pot tests were conducted in the greenhouse to determine the availability of the nitrogen in farm manure. One set of these tests was conducted with synthetic cow manure and horse manure made by mixing with straw the solid and liquid excrement in a definite proportion approximating that voided by the animal. The other test was made on dairy manure from the storage heap at the Marshfield substation. In the former case the start was made with fresh manure, and definite records were made on the storage and drying losses as well as the amount recovered in the four crops which were grown on the same pots over a period of two years.

This work was done in 2-gallon glazed earthenware pots. The soil was a

type intermediate between a sandy loam and a loamy sand very low in organic matter and nitrogen. All pots were supplied with sufficient lime, phosphorus, and potassium so as to eliminate all of these as factors. Each pot contained

TABLE 1

*Kinds of manure and amounts of nitrogen added*

| | POT NUMBER | TREATMENT | NITROGEN ADDED PER POT (10 KGM. SOIL) | | | |
|---|---|---|---|---|---|---|
| | | | Dung | Urine | Straw | Total |
| | | | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| Controls | 1–4 | | ... | ... | ... | ... |
| Cow manure, fresh | 5–6 | Dung only | 311.7 | ... | ... | 311.7 |
| | 7–8 | Dung and urine | 311.7 | 345.6 | ... | 657.3 |
| | 9–10 | Dung, urine, and straw | 311.7 | 345.6 | 74.9 | 732.2 |
| Cow manure, fresh, dried | 11–12 | Dung only | 290.2 | ... | ... | 290.2 |
| | 13–14 | Dung and urine | 290.2 | 179.2 | ... | 469.4 |
| | 15–16 | Dung, urine, and straw | 290.2 | 249.8 | 74.9 | 614.9 |
| Cow manure, fermented | 17–18 | Dung only | 289.4 | ... | ... | 289.4 |
| | 19–20 | Dung and urine | 289.4 | 298.8 | ... | 588.2 |
| | 21–22 | Dung, urine, and straw | 289.4 | 287.2 | 74.9 | 651.5 |
| Cow manure, fermented, dried | 23–24 | Dung only | 287.7 | ... | ... | 287.7 |
| | 25–26 | Dung and urine | 287.7 | 46.4 | ... | 334.1 |
| | 27–28 | Dung, urine, and straw | 287.7 | 74.3 | 74.9 | 436.9 |
| Cow manure, frozen dry | 29–30 | Dung only | 275.7 | ... | ... | 275.7 |
| | 31–32 | Dung and urine | 275.7 | 259.3 | ... | 535.0 |
| | 33–34 | Dung, urine, and straw | 275.7 | 314.6 | 74.9 | 665.2 |
| Horse manure, fresh | 35–36 | Dung only | 335.7 | ... | ... | 335.7 |
| | 37–38 | Dung and urine | 335.7 | 299.3 | ... | 635.0 |
| | 39–40 | Dung, urine, and straw | 335.7 | 299.3 | 74.9 | 709.9 |
| Horse manure, fresh, dried | 41–42 | Dung only | 317.5 | ... | ... | 317.5 |
| | 43–44 | Dung and urine | 317.5 | 194.0 | ... | 511.5 |
| | 45–46 | Dung, urine, and straw | 317.5 | 177.2 | 74.9 | 569.6 |
| Horse manure, fermented | 47–48 | Low straw, top | ... | ... | ... | 530.4 |
| | 49–50 | Low straw, bottom | ... | ... | ... | 652.7 |
| | 51–52 | High straw, top | ... | ... | ... | 516.0 |
| | 53–54 | High straw, bottom | ... | ... | ... | 535.2 |
| Horse manure, fermented, dried | 55–56 | Low straw, top | ... | ... | ... | 528.8 |
| | 57–58 | Low straw, bottom | ... | ... | ... | 360.6 |
| | 59–60 | High straw, top | ... | ... | ... | 538.0 |
| | 61–62 | High straw, bottom | ... | ... | ... | 336.0 |

10 kgm. of soil. The manure was applied at the rate of 12 tons an acre, 120 gm. a pot on the basis of the complete fresh manure. The manure was made up of 74 per cent by weight of dung, 20 per cent of urine, and 6 per cent of chopped straw. This was also true of the horse manure used, except that marked "high straw," in which 18 per cent of straw was used. Where the dung alone or dung and urine were added without straw, 74 per cent and 94 per cent respectively of the 120 gm. were added. If there were losses in storage or drying, this was deducted from the 120 gm. so that the application was always made on the fresh wet basis except for the fermented horse manure. Table 1 gives the treatments of the manures and the amounts of nitrogen added to the various pots.

The fermentation took place in 2-gallon stoneware containers at a temperature of 20°C. for four weeks. The tops of these containers were loosely covered to prevent excessive evaporation. The drying was done by spreading 500-gm. portions of the wet manure on glass plates in the greenhouse and allowing it to dry at 20°C. Complete air-drying took place in about five to six days. The manure was then removed from the plates and dried in the oven at 80°C. over night.

The manure frozen dry was spread on a glass plate and held at a temperature below freezing until dry. This required about two weeks. It was then dried in the oven.

Analyses were made both on the wet and dry samples. Wet analyses were made on 20-gm. samples in triplicate. After digestion, the samples were made up to 500 cc. and two-fifths of the sample was used. This procedure gave excellent results. It was found that the total nitrogen contained in manure could not be obtained after the samples were dried because of the loss in drying.

The manure was thoroughly incorporated with the moist soil by mixing and sifting. The plants were watered with distilled water. Four crops—oats, buckwheat, corn, and oats—were grown in rapid succession with no further additions of manure and with only from one to three months intervening between the crops. Table 2 gives yields and nitrogen content of those four crops. Each of these figures is the average of duplicate cultures.

None of these crops were grown to maturity but only to the point where they had used all of the available nitrogen. This came in the oats at about the heading stage, with the buckwheat when it was in blossom and with the corn when the first tassel appeared. The crops were then cut off at the surface of the soil and the roots left in the pot. The tops were oven-dried, weighed, ground, and the nitrogen determined. By deducting the nitrogen in the controls from that obtained from the other pots, the amount of nitrogen recovered from the manure is obtained. Table 3 shows these figures, together with the total amounts of nitrogen obtained in the four crops and the amounts added in the manure.

TABLE 2

*Yields of dry matter and nitrogen contents of the four crops: oats, buckwheat, corn and oats*

Amounts per pot (10 kgm. soil)

| | POT NUMBER | OATS, FIRST CROP | | BUCKWHEAT, SECOND CROP | | CORN, THIRD CROP | | OATS, FOURTH CROP | |
|---|---|---|---|---|---|---|---|---|---|
| | | Yield | Nitrogen | Yield | Nitrogen | Yield | Nitrogen | Yield | Nitrogen |
| | | *gm.* | *per cent* | *gm.* | *per cent* | *gm.* | *per cent* | *gm.* | *per cent* |
| Controls (average) | 1–4 | 7.60 | 0.98 | 5.1 | 1.19 | 9.65 | 0.98 | 3.45 | 1.44 |
| Cow manure, fresh | 5–6 | 7.50 | 1.15 | 6.6 | 1.15 | 10.65 | 0.95 | 4.25 | 1.46 |
| | 7–8 | 25.40 | 1.38 | 5.0 | 1.25 | 14.90 | 1.05 | 3.80 | 1.60 |
| | 9–10 | 22.50 | 1.35 | 4.1 | 1.29 | 19.40 | 0.95 | 3.75 | 1.68 |
| Cow manure, fresh, dried | 11–12 | 5.80 | 1.41 | 8.5 | 1.11 | 11.30 | 0.96 | 4.60 | 1.50 |
| | 13–14 | 14.40 | 1.15 | 4.. | 1.39 | 13.15 | 0.90 | 4.35 | 1.59 |
| | 15–16 | 13.10 | 1.21 | [illegible] | 1.38 | 14.15 | 0.85 | 4.35 | 1.76 |
| Cow manure, fermented | 17–18 | 8.00 | 1.22 | 8.3 | 1.20 | 14.75 | 0.90 | 4.90 | 1.53 |
| | 19–20 | 20.70 | 1.34 | 4.4 | 1.42 | 17.15 | 0.89 | 3.85 | 1.58 |
| | 21–22 | 19.60 | 1.26 | 6.6 | 1.24 | 12.95 | 1.03 | 5.75 | 1.46 |
| Cow manure, fermented, dried | 23–24 | 6.00 | 1.38 | 6.5 | 1.11 | 12.15 | 0.84 | 4.15 | 1.48 |
| | 25–26 | 6.70 | 1.22 | 5.5 | 1.16 | 10.80 | 0.94 | 4.85 | 1.44 |
| | 27–28 | 6.90 | 1.37 | 7.6 | 1.08 | 9.90 | 0.96 | 5.10 | 1.45 |
| Cow manure, frozen dry | 29–30 | 5.40 | 1.37 | 7.7 | 1.06 | 10.55 | 0.98 | 4.70 | 1.39 |
| | 31–32 | 16.90 | 1.31 | 4.6 | 1.27 | 12.65 | 1.14 | 4.65 | 1.35 |
| | 33–34 | 17.30 | 1.29 | 3.6 | 1.30 | 13.25 | 1.03 | 4.95 | 1.43 |
| Horse manure, fresh | 35–36 | 3.85 | 1.85 | 8.5 | 1.12 | 13.85 | 0.95 | 4.95 | 1.29 |
| | 37–38 | 20.00 | 1.34 | 4.1 | 1.44 | 16.00 | 1.04 | 5.40 | 1.28 |
| | 39–40 | 18.25 | 1.28 | 5.6 | 1.34 | 21.00 | 0.94 | 4.70 | 1.38 |
| Horse manure, fresh, dried | 41–42 | 2.95 | 1.96 | 8.6 | 1.04 | 14.55 | 0.79 | 5.15 | 1.27 |
| | 43–44 | 8.45 | 1.18 | 5.3 | 1.23 | 15.70 | 0.87 | 4.65 | 1.47 |
| | 45–46 | 6.40 | 1.47 | 8.4 | 1.06 | 15.55 | 0.90 | 5.80 | 1.35 |
| Horse manure, fermented | 47–48 | 8.05 | 1.33 | 8.8 | 1.16 | 15.30 | 0.95 | 5.30 | 1.32 |
| | 49–50 | 18.85 | 1.29 | 5.1 | 1.33 | 10.50 | 1.05 | 5.10 | 1.36 |
| | 51–52 | 9.95 | 1.24 | 7.7 | 1.16 | 16.45 | 0.86 | 5.20 | 1.31 |
| | 53–54 | 14.85 | 1.20 | 4.8 | 1.24 | 16.45 | 0.87 | 4.85 | 1.32 |
| Horse manure, fermented, dried | 55–56 | 5.95 | 1.47 | 7.4 | 1.19 | 12.50 | 0.83 | 6.00 | 1.23 |
| | 57–58 | 3.15 | 2.07 | 7.4 | 1.10 | 14.35 | 0.84 | 4.70 | 1.29 |
| | 59–60 | 8.35 | 1.13 | 4.4 | 1.32 | 15.95 | 0.87 | 4.95 | 1.36 |
| | 61–62 | 2.10 | 2.04 | 7.5 | 1.10 | 13.85 | 0.83 | 5.40 | 1.17 |

TABLE 3

*Nitrogen added in the manure and that recovered in the four crops: Oats, buckwheat, corn, and oats*

Amounts per pot (10 kgm. soil)

| | POT NUMBER | NITROGEN ADDED | | NITROGEN RECOVERED FROM MANURE BY FOUR CROPS | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Urine | Total | Oats | Buck-wheat | Corn | Oats | Total |
| | | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *mgm.* |
| Cow manure, fresh | 5–6 | ... | 311.7 | 11.7 | 15.2 | 6.7 | 20.0 | 53.6 |
| | 7–8 | 345.6 | 657.3 | 276.0 | 1.8 | 61.9 | 11.0 | 350.7 |
| | 9–10 | 345.6 | 732.2 | 229.2 | −2.8 | 70.8 | 13.3 | 305.5 |
| Cow manure, fresh, dried | 11–12 | ... | 290.2 | 7.3 | 33.7 | 14.0 | 19.3 | 74.3 |
| | 13–14 | 179.2 | 469.4 | 91.1 | 0.1 | 23.8 | 19.3 | 134.3 |
| | 15–16 | 249.1 | 614.9 | 84.0 | −9.6 | 26.8 | 26.8 | 128.0 |
| Cow manure, fermented | 17–18 | ... | 289.4 | 23.1 | 38.9 | 38.2 | 25.3 | 125.5 |
| | 19–20 | 298.8 | 588.2 | 202.9 | 1.8 | 58.1 | 11.3 | 274.1 |
| | 21–22 | 287.2 | 651.5 | 172.5 | 21.1 | 38.9 | 34.5 | 267.0 |
| Cow manure, fermented, dried | 23–24 | ... | 287.7 | 8.3 | 11.4 | 10.5 | 11.8 | 42.0 |
| | 25–26 | 46.4 | 334.1 | 7.2 | 3.1 | 7.0 | 20.0 | 37.3 |
| | 27–28 | 74.3 | 436.9 | 20.5 | 21.4 | 0.5 | 24.2 | 66.6 |
| Cow manure, frozen dry | 29–30 | ... | 275.5 | −0.5 | 21.9 | 9.0 | 15.7 | 46.1 |
| | 31–32 | 259.3 | 535.0 | 146.9 | −2.3 | 49.7 | 13.1 | 207.4 |
| | 33–34 | 314.6 | 665.2 | 148.7 | −13.9 | 43.3 | 21.3 | 199.4 |
| Horse manure, fresh | 35–36 | ... | 335.7 | −3.3 | 34.5 | 37.0 | 14.5 | 82.7 |
| | 37–38 | 299.3 | 635.0 | 194.5 | −1.6 | 72.0 | 19.4 | 284.3 |
| | 39–40 | 299.3 | 709.9 | 159.1 | 14.4 | 103.8 | 15.3 | 292.6 |
| Horse manure, fresh, dried | 41–42 | ... | 317.5 | −16.8 | 28.9 | 20.4 | 15.8 | 48.3 |
| | 43–44 | 194.0 | 511.5 | 25.2 | 4.5 | 32.8 | 18.5 | 81.0 |
| | 45–46 | 177.2 | 569.6 | 19.6 | 28.4 | 45.4 | 28.7 | 122.1 |
| Horse manure, fermented | 47–48 | Not | 530.4 | 34.2 | 41.4 | 50.8 | 20.1 | 146.5 |
| | 49–50 | known | 652.8 | 169.6 | 6.1 | 15.7 | 19.7 | 211.1 |
| | 51–52 | exactly | 516.0 | 48.9 | 20.5 | 46.9 | 18.7 | 135.0 |
| | 53–54 | | 535.2 | 103.8 | −1.1 | 48.5 | 14.3 | 165.5 |
| Horse manure, fermented, dried | 55–56 | Not | 528.8 | 13.0 | 22.5 | 9.2 | 24.0 | 68.7 |
| | 57–58 | known | 360.6 | −9.3 | 20.7 | 26.0 | 10.8 | 48.2 |
| | 59–60 | exactly | 538.0 | 19.9 | 2.6 | 45.0 | 17.9 | 80.2 |
| | 61–62 | | 336.0 | −31.7 | 21.8 | 20.4 | 13.4 | 23.9 |

Minus means less than the control.

A balance sheet showing the amounts of nitrogen lost in the various storing and handling operations is given in table 4. This table also shows the percentage recoveries from the various treatments based both on the original total and also on the original amount of liquid manure nitrogen present.

Losses of nitrogen in the handling of manure occur at two points; first, during the process of storage, and second, in the process of spreading and exposure before the manure is incorporated with the soil. The figures in table 4 show a fermentation loss of 7 to 11 per cent. This will vary directly with the exposure, temperature, and evaporation. If the manure is in a deep, moist heap and well packed, only slightly exposed at the top and with no drainage, the loss from storage will not be more than a few per cent, which comes largely during the ammonification period. These data correspond to the findings of other investigators already cited.

The second and perhaps greatest loss of the nitrogen from farm manure comes during the drying period from the time the manure is spread in the field until it is plowed under. These handling, spreading, and drying losses vary greatly with the conditions, being greater with the higher temperature and more excessive drying. The losses given in table 4 for spreading and drying would be the maximum that could possibly occur. The loss of nitrogen from the dung by drying is always small, whatever the previous treatment may have been. This would be expected because practically all of the dung nitrogen is organic and only about 20 per cent of it is changed to ammonia in storage.

The greatest loss of nitrogen comes from the urine or liquid manure. When the liquid is mixed with the dung only and the fresh material allowed to dry, there is a loss of 28.6 per cent of the total nitrogen, or about twice that percentage of the liquid manure nitrogen. This loss comes largely because the manure does not dry fast enough to prevent ammonification and its consequent loss. The low temperature of the frozen manure gives a lesser loss, due perhaps for the most part to the lack of ammonification. The drying losses are much greater in the fermented manure because the liquid manure nitrogen is entirely changed to ammonia and held in solution as salts of volatile acids. The figures show a loss under these conditions of from 30 to 45 per cent of the total nitrogen, which is approximately from about 50 to 90 per cent of the liquid manure nitrogen. One very outstanding fact in connection with the fermented horse manure is the difference in the drying loss between the top and bottom of the heap. From the manure in the top 5 or 6 inches of the heap there is little or no loss of nitrogen on drying, but when the manure from the bottom portion of the heap is dried, from 80 to 90 per cent of its liquid manure nitrogen is lost by volatilization. This is explained by the fact that the liquid manure nitrogen in the bottom of the heap (strictly anaerobic fermentation) is entirely in the form of ammonium salts of volatile acids and is lost easily, whereas the nitrogen in the top part has passed through an aerobic decomposition, has lost some of its ammonia during the process and the rest has been changed to an organic form by microörganisms, and is not volatile on drying.

TABLE 4

*Losses and recovery of nitrogen by the four crops from 120 gm. of manure or its component parts in the various treatments*

Amounts per pot (10 kgm. soil)

| | POT NUMBER | ORIGINAL NITROGEN IN MANURE | | NITROGEN LOST IN STORAGE (FERMENTATION) | | NITROGEN LOST IN HANDLING (DRYING) | | NITROGEN RECOVERED IN 4 CROPS | | | NITROGEN LOST PLUS THAT RECOVERED IN 4 CROPS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Urine | Total | | | | | Original total | | Original urine | | |
| | | *mgm.* | *mgm.* | *mgm.* | *per cent* | *mgm.* | *per cent* | *mgm.* | *per cent* | *per cent* | *mgm.* | *per cent* |
| Cow manure, fresh | 5–6 | ... | 311.7 | ... | ... | ... | ... | 53.6 | 17.2 | ... | 53.6 | 17.2 |
| | 7–8 | 345.6 | 657.3 | ... | ... | ... | ... | 350.7 | 54.3 | 101.6 | 350.7 | 54.3 |
| | 9–10 | 345.6 | 732.2 | ... | ... | ... | ... | 305.5 | 41.7 | 88.5 | 305.5 | 41.7 |
| Cow manure, fresh, dried | 11–12 | ... | 311.7 | ... | ... | 21.5 | 6.9 | 74.3 | 23.8 | ... | 95.8 | 30.7 |
| | 13–14 | 345.6 | 657.3 | ... | ... | 187.9 | 28.6 | 134.3 | 20.4 | 38.9 | 322.2 | 49.0 |
| | 15–16 | 345.6 | 732.2 | ... | ... | 117.3 | 16.0 | 128.0 | 17.5 | 37.1 | 245.3 | 31.5 |
| Cow manure, fermented | 17–18 | ... | 311.7 | 22.3 | 7.16 | ... | ... | 125.5 | 40.3 | ... | 147.8 | 47.5 |
| | 19–20 | 345.6 | 657.3 | 69.1 | 10.5 | ... | ... | 274.1 | 41.7 | 79.4 | 343.2 | 52.2 |
| | 21–22 | 345.6 | 732.2 | 80.7 | 11.2 | ... | ... | 267.0 | 36.5 | 77.3 | 347.7 | 47.7 |
| Cow manure, fermented, dried | 23–24 | ... | 311.7 | 22.3 | 7.16 | 1.7 | 0.54 | 42.0 | 13.5 | ... | 76.0 | 21.2 |
| | 25–26 | 345.6 | 657.3 | 69.1 | 10.5 | 254.1 | 38.7 | 37.3 | 5.6 | 10.8 | 360.5 | 54.8 |
| | 27–28 | 345.6 | 732.2 | 80.7 | 11.2 | 214.6 | 29.3 | 66.6 | 9.1 | 19.3 | 361.9 | 49.6 |
| Cow manure, frozen dry | 29–30 | ... | 311.7 | ... | ... | 36.0 | 11.5 | 46.1 | 14.8 | ... | 82.1 | 26.3 |
| | 31–32 | 345.6 | 657.3 | ... | ... | 121.4 | 18.5 | 207.4 | 31.5 | 60.0 | 328.8 | 50.0 |
| | 33–34 | 345.6 | 732.2 | ... | ... | 67.0 | 9.1 | 199.4 | 27.2 | 52.7 | 266.4 | 36.3 |
| Horse manure, fresh | 35–36 | ... | 335.7 | ... | ... | ... | ... | 82.7 | 24.6 | ... | 83.7 | 24.6 |
| | 37–38 | 299.3 | 635.0 | ... | ... | ... | ... | 284.3 | 44.8 | 95.0 | 284.3 | 44.8 |
| | 39–40 | 299.3 | 709.9 | ... | ... | ... | ... | 292.6 | 41.2 | 97.7 | 292.6 | 41.2 |
| Horse manure, fresh, dried | 41–42 | ... | 335.7 | ... | ... | 18.2 | 5.4 | 48.3 | 14.4 | ... | 66.5 | 19.8 |
| | 43–44 | 299.3 | 635.0 | ... | ... | 123.5 | 19.4 | 81.0 | 12.7 | 27.0 | 204.5 | 32.1 |
| | 45–46 | 299.3 | 709.9 | ... | ... | 140.3 | 19.8 | 122.1 | 17.2 | 40.8 | 263.4 | 37.0 |
| Horse manure, fermented | 47–48 | Not | 530.4 | ... | ... | ... | ... | 146.5 | 27.6 | ... | 146.5 | 27.6 |
| | 49–50 | known | 652.8 | ... | ... | ... | ... | 211.1 | 31.1 | ... | 211.1 | 31.1 |
| | 51–52 | exactly | 516.0 | ... | ... | ... | ... | 135.0 | 26.2 | ... | 135.0 | 26.2 |
| | 53–54 | | 535.2 | ... | ... | ... | ... | 165.5 | 30.9 | ... | 165.5 | 30.9 |
| Horse manure, fermented, dried | 55–56 | Not | 530.4 | ... | ... | 1.6 | 0.3 | 68.7 | 13.0 | ... | 70.3 | 13.3 |
| | 57–58 | known | 652.8 | ... | ... | 292.2 | 44.8 | 48.2 | 7.4 | ... | 340.4 | 52.2 |
| | 59–60 | exactly | 516.0 | ... | ... | No loss | | 80.2 | 15.6 | ... | 80.2 | 15.6 |
| | 61–62 | | 535.2 | ... | ... | 199.2 | 37.2 | 23.9 | 4.5 | ... | 223.1 | 41.7 |

Straw as bedding in the manure tends to reduce the drying losses and the more straw used, the greater is the saving of nitrogen. Although straw shows up as an aid in preventing loss at this point, it is a decided detriment to the availability of the nitrogen, which is inversely proportional to the amount of straw present. In this case the straw furnishes energy material for the growth of soil organisms, especially fungi, which compete with the plant in the use of nitrogen and cause a lower recovery of nitrogen in the crop. Allison (1) and many other workers have shown that the energy materials supplied in manure have this effect.

The recovery of the manure nitrogen by the four crops varied from almost nothing to over 50 per cent of the total nitrogen or approximately 100 per cent of the liquid manure nitrogen. The availability of the dung nitrogen is low

TABLE 5

*Analysis of stored solid and liquid manure from the Marshfield and Hancock (Wisconsin) substations*

| | COMPLETE MANURE STORED IN THE HEAP | LIQUID MANURE FROM THE CISTERN |
|---|---|---|
| Marshfield, 1928: | | |
| Dry matter, per cent | 23.3 | ... |
| Specific gravity | ... | 1.0134 |
| Total nitrogen, per cent (wet) | 0.56 | 0.33 |
| Total nitrogen, per cent (dry) | 1.97 | ... |
| Nitrogen lost on drying, per cent (wet) | 0.10 | ... |
| Total phosphorus, per cent (wet) | 0.115 | ... |
| Total potassium, per cent (wet) | ... | 0.426 |
| Hancock, 1929: | | |
| Total nitrogen, per cent (wet) | 0.665 | 0.35 |
| Ammonia nitrogen, per cent (wet) | 0.183 | 0.33 |
| Potassium, per cent (wet) | ... | 0.336 |

except in case of the fermented dung, where it reaches 40 per cent of the nitrogen, but in most cases is below 20 per cent. With the cow manure, where the dung and liquid alone are used, the amount of nitrogen recovered in the crop is the difference between the total liquid manure nitrogen and the losses occurring before the manure is incorporated into the soil. If two-thirds of the liquid manure nitrogen is lost in storage and handling, only one-third is recovered in the crops. The availability of the nitrogen in horse manure is usually a little lower than that from cow manure; first, because there is usually a lesser proportion of liquid manure nitrogen present, and, second, there is more energy material of a cellulose nature. Straw nearly always lowers the availability in direct proportion to the amount present. Manure decomposed aerobically and well rotted shows a lower availability than the anaerobically fermented manure because of the change from ammonia to organic nitrogen in the aerobic decomposition.

*Tests with Manure from the Marshfield Substation.* Manures from the storage heap and liquid manure cistern at the Marshfield substation were also used in pot tests. Table 5 shows the composition of these manures from Marshfield for 1928 and also from the Hancock substation for 1929.

The composition of these manures is very different from that of the synthetic manures used in the previous tests. Although the total nitrogen in the manure

TABLE 6

*Application and recovery of nitrogen from manure from the Marshfield substation applied to fallow pots*

Amounts per pot (10 kgm. soil)

| POT NUMBER | TREATMENT | MANURE APPLIED | NITROGEN ADDED IN MANURE | NITRATE NITROGEN RECOVERED FROM FALLOW | | |
|---|---|---|---|---|---|---|
| | | | | Total | From original manure | |
| | | *grams* | *mgm.* | *mgm.* | *mgm.* | *per cent* |
| 1 | Control, no manure | ... | ... | 130 | ... | ... |
| 2 | Manure fresh from heap—turned under immediately | 120 | 672 | 340 | 210 | 31.3 |
| 3 | Manure fresh from heap—wet down 3 or 4 times, allowed to dry, then turned under | 120 | 672 | 310 | 180 | 26.8 |
| 4 | Manure dried before application | 28 (dry) | 552 | 160 | 30 | 5.6 |
| 5 | Liquid manure, turned under immediately | 30.4 | 100 | 240 | 110 | 110.0 |

TABLE 7

*Recovery of nitrogen by corn from manure from the Marshfield substation*

Amounts per pot (10 kgm. soil)

| POT NUMBER | WEIGHT OF DRY MATERIAL | | | NITROGEN IN DRY MATERIAL | | | | | NITROGEN ADDED IN MANURE | RECOVERY OF ORIGINAL MANURE NITROGEN ADDED | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tops | Roots | Total | Tops | | Roots | | Total | | | |
| | *grams* | *grams* | *grams* | *per cent* | *mgm.* | *per cent* | *mgm.* | *mgm.* | *mgm.* | *mgm.* | *per cent* |
| 1 | 10.5 | 7.5 | 18.0 | 0.77 | 80.8 | 0.92 | 69.0 | 148.8 | ... | ... | ... |
| 2 | 25.3 | 9.7 | 35.0 | 0.97 | 245.4 | 1.01 | 98.0 | 343.4 | 672 | 194.6 | 28.9 |
| 3 | 22.6 | 11.0 | 33.6 | 0.86 | 194.4 | 0.93 | 102.3 | 296.7 | 672 | 147.9 | 22.0 |
| 4 | 15.5 | 10.3 | 25.8 | 0.73 | 113.1 | 0.86 | 88.6 | 201.7 | 552 | 52.9 | 9.5 |
| 5 | 19.4 | 10.5 | 29.9 | 0.80 | 155.2 | 0.86 | 90.3 | 245.5 | 100 | 96.5 | 96.5 |

is high, as much as 13 pounds of nitrogen to a ton of manure, the ammonia portion is very low. This condition may be due to the failure to incorporate all of the liquid into the storage heap or its subsequent loss by leaching, or it may be due to a more or less aerobic condition of the heap and a change from ammonia to organic nitrogen or even some losses of ammonia nitrogen.

Pot tests with the manure from Marshfield were made as already outlined.

The complete manure was applied at the rate of 12 tons an acre and the liquid manure 3 tons an acre. Four pots of each treatment were made, two of which were kept in clean fallow and two planted to corn. The corn was grown to the tasseling stage, harvested, the roots removed from the soil, and both top and roots were dried, weighed, and analyzed. The fallow pots were carried along until the harvest of the corn when the amount of nitrate nitrogen was determined in them. Table 6 shows the treatments, amounts of nitrogen applied to all pots, and the nitrogen recovered as nitrate from the fallow pots. Table 7 gives the yields of corn, its nitrogen content, and the nitrogen recovered from the manure by the corn.

It is interesting to note that the amounts of nitrogen recovered in the corn are approximately the same as those obtained as nitrate from the manure in the fallowed pots. In this case, moisture was not a factor and it would be logical to expect as much nitrification in one case as in the other. With this particular manure, less than 30 per cent of its total nitrogen is recovered in the total crop when the manure is incorporated in the soil at once with no loss of nitrogen. This indicates that between 30 and 50 per cent of the liquid manure nitrogen either was not incorporated into the storage heap or was lost by leaching or volatilization in the storage process. When the manure is wet down after spreading, in imitation of rain, and a part of the soluble nitrogen washed into the soil before the manure is allowed to dry prior to plowing down, there is a recovery of less than 25 per cent of the total nitrogen, thus showing an additional loss from the drying after the rain of 5 to 7 per cent of the total nitrogen. If the manure is dried completely before application, the availability is reduced to almost nothing, showing that practically all of the ammonia nitrogen is lost when the manure dried at 80°C. Figures in table 10 show that 95 per cent of the ammonia nitrogen from fermented manure is lost by drying in this way and this corresponds closely to the availability to corn of this manure nitrogen.

When the liquid manure is used alone its nitrogen is practically all recovered in the crop. This nitrogen is completely water-soluble and largely in the ammonia form with no energy material present. This liquid manure, however, was very low in nitrogen, carrying only 10 pounds a ton. To apply 30 pounds of nitrogen to the acre in this form it would be necessary to apply 3 tons, or about 750 gallons, of the liquid to the acre.

## STORAGE AND HANDLING CHANGES IN MANURE; LABORATORY ANALYSES

*Changes in storage.* A series of laboratory studies were made to determine the nature of the changes taking place in manure when stored and how these changes affect the losses in handling. A series of closed containers were set up and filled with urine, dung, and mixtures of these, together with straw. The mixtures were made up on the basis of dung 74 per cent, urine 20 per cent, and straw 6 per cent for the complete manure. The tops of these containers were closed and the air space above the manure filled with carbon dioxide more

nearly to imitate anaerobic conditions in the compact heap. A mercury seal was placed on each container. These containers were held at 20°C. for 30 days and then determinations were made for total and ammonia nitrogen, volatile and non-volatile acids, and pH. Table 8 gives these data.

The urine nitrogen changes almost completely to ammonia, only about 5 per cent remaining in organic form. Because of the large amount of ammonia formed, the pH value goes up to 9.4 at the end of 30 days. This change is complete in a short time with practically no subsequent change.

TABLE 8

*The composition and reaction of urine, dung, and cow manure mixtures on standing*

| | URINE ONLY | DUNG ONLY | DUNG AND URINE<br>Dung: 78.7%<br>Urine: 21.3% | COMPLETE MANURE<br>Dung: 74%<br>Urine: 20%<br>Straw: 6% |
|---|---|---|---|---|
| Nitrogen in urine, per cent | 1.106 | ..... | 1.106 | 1.106 |
| Nitrogen in dung, per cent | ..... | 0.386 | 0.386 | 0.386 |
| Nitrogen in straw, per cent | ..... | ..... | ..... | 1.04 |
| Urine nitrogen in mixture, per cent | 1.106 | ..... | 0.2356 | 0.2212 |
| Dung nitrogen in mixture, per cent | ..... | 0.386 | 0.3038 | 0.2856 |
| Straw nitrogen in mixture, per cent | ..... | ..... | ..... | 0.0624 |
| Total nitrogen in mixture at beginning, per cent | 1.106 | 0.386 | 0.5394 | 0.5692 |
| After 30 days: | | | | |
| Total nitrogen, per cent | 1.077 | 0.402 | 0.525 | 0.575 |
| Ammonia nitrogen, per cent | 1.018 | 0.084 | 0.278 | 0.271 |
| Volatile acids per gram of manure, cc. $N/14$ acid | ..... | 2.20 | 2.772 | 4.295 |
| Non-volatile acids per gram of manure, cc. $N/14$ acid | ..... | 1.12 | 0.784 | 0.9075 |
| pH | 9.4 | 5.27 | 7.37 | 6.79 |
| After 60 days: | | | | |
| Total nitrogen, per cent | ..... | ..... | ..... | 0.574 |
| Ammonia nitrogen, per cent | ..... | ..... | ..... | 0.296 |

In 30 days only about 20 per cent of the dung nitrogen is changed to ammonia. This material is acid at the end of this period with a pH value of 5.27 and volatile acids equivalent to 2.2 cc. of $N/14$ acid per gram of manure, or nearly three times the amount of acid necessary to unite with the ammonia nitrogen present. This is the most acid fermentation because it contains no urine and consequently little ammonia nitrogen is produced to raise the pH value. After 30 days a little over half of the nitrogen in the dung and urine mixture is ammonia. The volatile acids in this mixture are equivalent to 2.77

cc. of $N/14$ acid per gram of manure, a little increase over the dung alone, but the pH value is up to 7.37 because of the large amount of ammonia present. When straw is added to the dung and urine there is an increase in the amount of volatile acids and a drop in the pH value. The addition of straw has practically no effect upon the ammonification process or the amount of ammonia produced, but the energy material in the straw materially increases the amount of volatile acid produced. At 60 days there is a slight increase in the ammonia content.

Ammonia traps were placed inside of each of these containers and, with the exception of the liquid alone, there is practically no volatilization of ammonia nitrogen. It seems that loss of nitrogen as ammonia from manure mixtures is always accompanied by evaporation.

TABLE 9

*Changes in the nitrogen content of fermented manure exposed to drying*

Amounts from 20-gm. sample

| | AMMONIA NITROGEN | NON-AMMONIA NITROGEN | |
|---|---|---|---|
| | | Water-soluble | Water-insoluble |
| | *mgm.* | *mgm.* | *mgm.* |
| Atmosphere still—no wind: | | | |
| 12 hours—20°C. | 50.2 | 13.0 | 47.8 |
| 36 hours—20°C. | 32.0 | 10.7 | 43.6 |
| 3½ days—20°C. | 21.4 | 11.65 | 39.8 |
| 7 days—20°C. | 17.0 | 10.4 | 41.6 |
| 7 to 8 days*—80°C. | 3.0 | 8.1 | 45.4 |
| Atmosphere moving—8½-mile wind: | | | |
| 12 hours—20°C. | 30.0 | 11.8 | 40.8 |
| 36 hours—20°C. | 23.1 | 12.1 | 40.3 |
| 3 ½days—20°C | 17.7 | 12.6 | 40.6 |
| 7 days—20°C. | 15.7 | 11.4 | 40.4 |
| 7 to 8 days*—80°C. | 2.85 | 8.5 | 43.4 |
| In manure before exposure | 59.2 | 57.5 | |

* See note, table 10.

*Losses of manure nitrogen in handling.* A study was made of the losses of nitrogen from the complete manure mixture of dung, urine, and straw shown in table 8. Samples of a given weight were spread out on glass plates at the rate of 12 tons an acre. They were allowed to stand at room temperature (20°C.) and humidity. One set was exposed to a still atmosphere and another set to air moving at the rate of 8½ miles an hour. They were not exposed to sunshine. At varying intervals of time, the ammonia nitrogen and non-ammonia nitrogen were determined. The last samples, after standing at room temperature for 7 days, were heated at 80°C. for 24 hours. The ammonia

was determined by distilling the water-soluble fraction of the manure with magnesium oxide. These data are given in table 9.

There is very little change in the non-ammonia nitrogen but the ammonia nitrogen is almost completely lost under the conditions of the experiment. Table 10 gives the losses of ammonia nitrogen in per cent based both on the total and the ammonia nitrogen. From these data it may be seen that in seven days nearly three-fourths of the ammonia nitrogen in fermented manure is lost by volatilization. When the atmosphere is in motion, the loss is much more rapid in the beginning, but at the end of a 7-day period, approximately the same amounts are lost. Heating to 80°C. causes a further loss of about 20 per cent and leaves only a 5 per cent residue of ammonia nitrogen. The 8½-mile wind causes a greater loss in 12 hours than is lost from the samples dried without wind in 36 hours. Almost one-half of the ammonia nitrogen is lost in 12 hours with the 8½-mile wind. The loss of nitrogen from the drying

TABLE 10

*Losses of ammonia-nitrogen from fermented manure exposed to drying*

Amounts from 20-gm. sample

| AFTER | ATMOSPHERE STILL—NO WIND | | | ATMOSPHERE MOVING—8½ MILE WIND | | |
|---|---|---|---|---|---|---|
| | Total nitrogen | | Ammonia nitrogen | Total nitrogen | | Ammonia nitrogen |
| | *mgm.* | *per cent* | *per cent* | *mgm.* | *per cent* | *per cent* |
| 12 hours—20°C. | 9.0 | 7.7 | 15.2 | 29.2 | 25.1 | 49.4 |
| 36 hours—20°C. | 27.2 | 23.4 | 46.0 | 36.1 | 30.9 | 61.0 |
| 3½ days—20°C. | 37.8 | 32.4 | 63.9 | 41.5 | 35.6 | 70.0 |
| 7 days—20°C. | 42.2 | 36.2 | 71.3 | 43.5 | 37.3 | 73.5 |
| 7 to 8 days*—80°C. | 56.2 | 48.2 | 95.0 | 56.4 | 48.3 | 95.2 |

* After air-drying in the open for 7 days the residue was placed in the 80° oven for 24 hours.

fermented manure seems to take place in two stages. In the first stage the loss is very rapid and uniform and this stage covers approximately one-half of the ammonia nitrogen. In the second stage the loss is much slower and represents about one-half of the remaining ammonia nitrogen.

A further study of the volatile substances given off from manure was made in an attempt to explain the reason for the volatilization of the ammonia nitrogen. A small container 10 inches by 2 inches by ½ inch deep was arranged so that 30 gm. of manure could be spread on the bottom at the rate of 12 tons an acre and then carbon dioxide- and ammonia-free air was drawn over the manure and the ammonia, carbon dioxide, and volatile acids were trapped and determined. This was carried on for 144 hours in three 48-hour periods. The results are given in table 11.

These data, together with those in table 10, show that the loss of ammonia nitrogen proceeds up to the extent of about 50 per cent largely as the carbonate,

with perhaps little loss as the salts of volatile acids. This loss, taking place while the manure is still moist, is very rapid and follows almost a straight line. When the moisture is gone, the loss of nitrogen is for the most part ammonium salts of the volatile acids and is much slower. These volatile acids are in all

TABLE 11

*Ammonia nitrogen, carbon-dioxide carbon, and volatile acids lost in 144 hours from drying fermened manure*

Amounts from 30-gm. sample of complete manure

| | AMMONIA NITROGEN | CARBON-DIOXIDE CARBON | VOLATILE ACIDS |
|---|---|---|---|
| | *mgm.* | *mgm.* | *cc. N/14 acid* |
| First 48 hours | 51.8 | 38.4 | 10.5 |
| Second 48 hours | 11.2 | 6.0 | 10.5 |
| Third 48 hours | 6.8 | 7.8 | 4.2 |
| Total volatilized in 144 hours | 69.8 | 52.2 | 25.2 |
| Total ammonia nitrogen in sample | | | 88.8 |
| Total nitrogen in sample | | | 172.2 |

TABLE 12

*Losses of nitrogen from liquid manure under different methods of storage*

| | OPEN—NO TREATMENT | OPEN—50 GM. 45 PER CENT SUPERPHOSPHATE | OPEN—¼ INCH OIL COVER |
|---|---|---|---|
| At beginning: | | | |
| Amount used, cc | 700 | 700 | 680 |
| Specific gravity, liquid | 1.03 | 1.03 | 1.03 |
| Total nitrogen, per cent | 1.29 | 1.29 | 1.29 |
| At end of 30 days: | | | |
| Loss in weight, gm | 40 | 32 | 0 |
| Ammonia nitrogen, per cent | 0.952 | 0.998 | 1.242 |
| Total nitrogen, per cent | 1.039 | 1.159 | 1.277 |
| Weight liquid manure, gm | 681 | 689 | 700.4 |
| Weight of nitrogen in manure, gm | 6.94 | 7.98 | 8.99 |
| Loss of nitrogen, gm | 2.38 | 1.34 | 0.07 |
| Loss of nitrogen, per cent | 25.5 | 14.37 | 1.08 |
| At end of 6 months: | | | |
| Loss of nitrogen, per cent | .... | .... | 1.27 |

probability largely acetic and butyric. These acids were isolated as a group and gave a Duclaux curve similar to the lower organic acids, but no definite identifications were made.

*Storage losses from liquid manure.* Three flasks of fresh urine were set up in the laboratory and held at 20°C. They were left unstoppered. No. 1 was

held as a control with no treatment, no. 2 received 50 gm. of 45 per cent superphosphate, and no. 3 received no chemical treatment but was covered with a layer of lubricating oil ¼ inch deep. They were allowed to stand for 30 days and analyses made of their contents. Table 12 shows the results of this study.

The control lost 25.5 per cent of its nitrogen in 30 days; the phosphate-treated urine, 14.4 per cent; and the oil-covered urine, only 1 per cent in 30 days and only 1.27 per cent after a period of 6 months. If urine is treated with a mineral acid or a soluble salt of a mineral acid, especially a calcium salt, the ammonia in the liquid manure is changed to the salt of a non-volatile acid and is not lost from the solution. None of these chemical treatments have proved practical for farm use, either because of the expense or some other disadvantage, such as the insoluble residue left by the superphosphate, which must be removed fom the cistern in the solid form.

The layer of oil, however, is a very efficient and cheap means of conserving the liquid manure nitrogen. From a practical standpoint, used crank case oil can be obtained at a very low cost and the saving of nitrogen is almost 100 per cent. The delivery pipe and the pump can be run to the bottom of the cistern and the liquid manure delivered and pumped out from under this oil layer, which forms a perfect seal. Losses of nitrogen on spreading may be reduced by dilution before spreading or by drilling the liquid under the surface of the soil.

## DISCUSSION

Farm manure may be applied to the land as soon as it is produced or it may be stored until some convenient time in the cropping system. Either method may be used with advantage at times, and if handled properly, with a minimum loss of nitrogen, but perhaps by far the greater portion of farm manure is stored before application. For general livestock or dairy farming this is probably the simplest and best adapted. Although European farmers have used the separate storage to advantage in their more intensive farming, it is a question whether it will ever come into general use in the more extensive American system. It seems that for the more extensive farmer the storage of the dung, urine, and bedding together is most convenient. This storage should accomplish three things; namely, the incorporation of all of the liquid excrement with the manure, the prevention of any leaching, and the maintenance of anaerobic conditions in the storage heap. The first is accomplished by the use of gutters for the prevention of loss, and the use of sufficient absorbent or bedding. Leaching in storage is best prevented by the use of some sort of concrete container with a tight bottom so that no liquid may be lost. It is not necessary for this manure container to be covered, in fact unless the rainfall is very high it is better for the manure to receive the normal precipitation, as long as there is no leaching. This precipitation insures the necessary moisture to produce and maintain anaerobic conditions and these conditions are better produced by packing the normally moist manure than by water-logging. There is

enough moisture in cattle manure so that if it is packed down, anaerobic conditions will result. European farmers are careful of this point and often use animals for the packing. This stored manure quickly ammonifies and then in the course of a month or two, depending upon the temperature, goes through a fermentation process similar to that of silage fermentation. If this storage is done properly the end product will contain practically all of the nitrogen in the original dung and urine, and about half of it, or that portion originally in the urine, will be in the form of ammonium carbonate and the ammonium salts of volatile organic acids. If this process is properly done, the loss of nitrogen in storage of manure should be less than 10 per cent of the original nitrogen.

The handling and spreading of anaerobically stored manure must be done properly if minimum losses are to be obtained. Three-eights to one-half of the nitrogen in this manure is easily lost by exposure to drying conditions. Greatest spreading losses occur when weather conditions are best for drying. Warm, dry, sunny days with wind produce conditions which cause greatest losses. If the spreading is done during cool, rainy weather and the manure incorporated with the soil immediately, the losses may be reduced to a minimum. The handling of fermented manure is made much easier if cut straw is used for liter. The spreading of fresh manure is attended by the loss of less nitrogen than where the manure is fermented. The practice of applying the fresh manure to snow-covered fields in winter gives low handling losses, but on the other hand, if fresh manure is spread and allowed to lie on the land during warm sunny weather, ammonification takes place within two or three days and the losses may be heavy.

The available nitrogen in farm manure is largely confined to the ammonia or water-soluble portions. The water-soluble nitrogen in farm manure, minus the losses in storage and handling, gives a very good measure of the nitrogen in manure which is readily obtained by the growing crop. Very little of the dung nitrogen is readily utilized by crops. Only in the case of fermented dung does any appreciable amount of the dung nitrogen become available. The amount of the nitrogen in the liquid furnishes a very good measure of the value of the nitrogen in farm manure. When straw is used for bedding to absorb the liquid, it not only acts as an absorbent, but also aids in decreasing the losses on drying after the manure is spread. Although the straw is an advantage in this respect, it is a decided disadvantage in the amount of manure nitrogen recovered in the crop. The straw furnishes energy for the growth of soil microorganisms with a resultant depression in the nitrate and available nitrogen. As a result, the greater amount of straw used as bedding, the greater the depression in the availability of the manure nitrogen. This depression is especially marked in the first crop following the manure application.

## SUMMARY

This paper reports a laboratory and greenhouse study of the changes, losses, and availability of the nitrogen in farm manure. The results may be summarized as follows:

When manure is stored under anaerobic conditions two changes take place; namely, the urea and water-soluble nitrogen change to ammonia, and an acid fermentation takes place with the formation of volatile organic acids which react with the ammonia, forming salts.

The losses of nitrogen from farm manure come both in the storage and handling processes. Under extreme conditions these losses combined may be almost one-half of the total nitrogen or nearly the whole of the liquid manure nitrogen. The loss of nitrogen is due to the volatilization of ammonia either as the carbonate or as the salt of a volatile acid. The former seems to be lost most rapidly during the early drying period and the latter when most of the carbonate is gone. The ammonification process results in an increase in the pH value and the fermentation process causes the pH value to decrease, and this decrease is in direct proportion to the amount of straw or energy material present.

When liquid manure is stored alone, the nitrogen changes to ammonium carbonate and is lost from the solution by volatilization. This loss may be reduced to almost nothing by covering the surface of the liquid with a thin layer of mineral oil. Although the container may be open, there is practically no loss of nitrogen through the oil layer.

Straw as bedding in the manure tends to reduce the drying losses of nitrogen, but at the same time it reduces the availability of manure nitrogen.

An approximate measure of the nitrogen available from farm manure is obtained by the difference between the water-soluble or ammonia nitrogen and the losses of nitrogen from the manure during storage and handling. If there is no loss of nitrogen from cattle manure, 50 per cent of the total, or 100 per cent of the liquid, manure nitrogen should be recovered in the first crop under normal conditions. A smaller recovery than this is probably due either to loss of liquid manure nitrogen or the use of excessive amounts of straw as bedding.

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## PLATE 1

GROWTH OF OATS ON SOILS VARIOUSLY TREATED

FIG. 1. Comparative growth of oats on the control and also on the dung alone from cow manure treated in various ways.

FIG. 2. Comparative growth of oats on fresh cow manure and the same after being dried (Pots 5 and 11 are dung alone; 7 and 13 are dung and urine; and 9 and 15 are dung, urine, and straw).

FIG. 3. Comparative growth of oats on fermented cow manure and the same after being dried (Pots 17 and 23 are dung alone; 19 and 25 dung and urine; and 21 and 27 dung, urine, and straw).

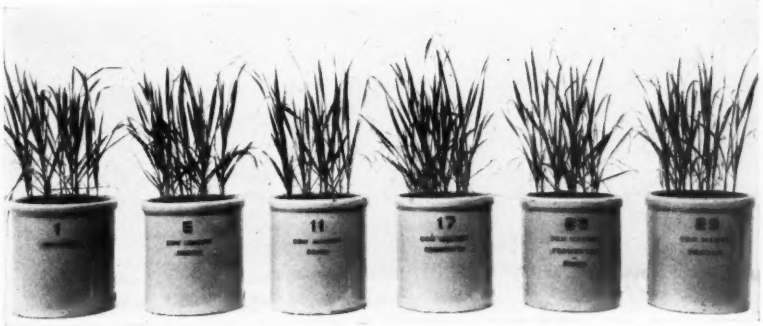

FIG. 1

FIG. 2

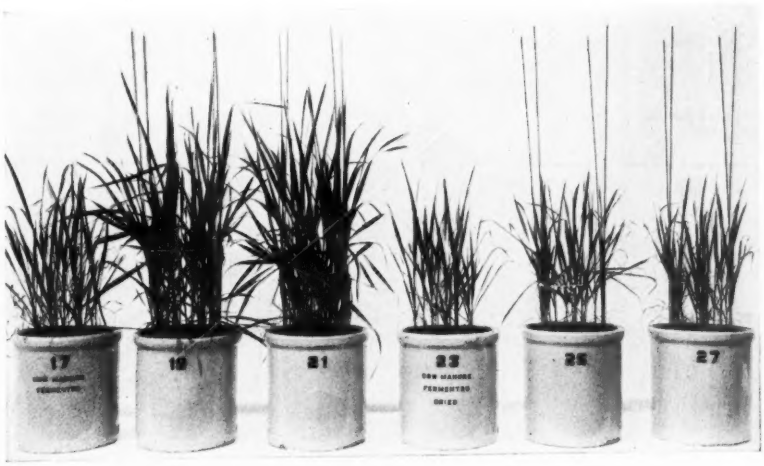


FIG. 3

## PLATE 2

GROWTH OF BUCKWHEAT ON SOILS VARIOUSLY TREATED

FIG. 1. Comparative growth of buckwheat following oats on the same pots shown in plate 1, figure 2.

FIG. 2. Comparative growth of buckwheat following oats on the same pots shown in plate 1, figure 3.

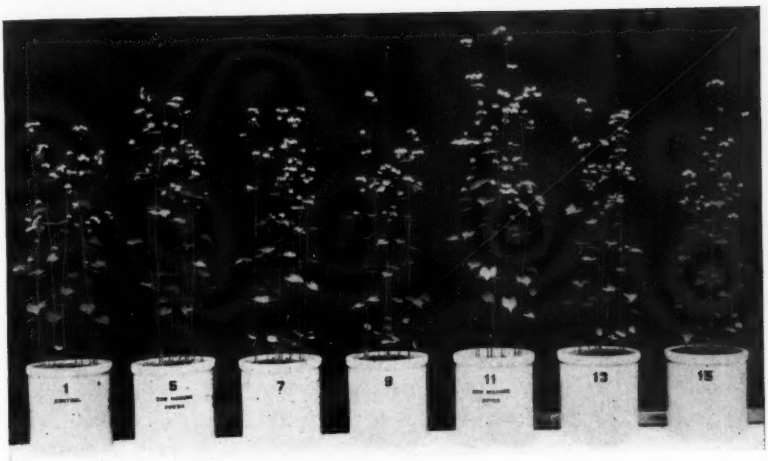


FIG. 1

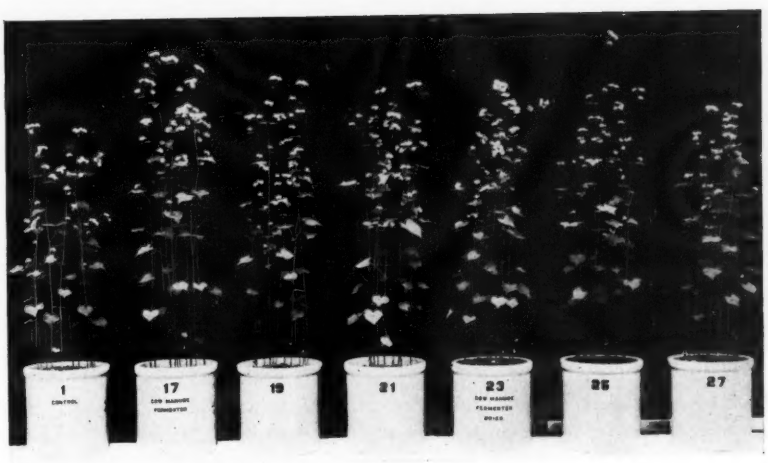


FIG. 2

# THE INTERRELATIONSHIPS OF CERTAIN SINGLE-VALUED SOIL PROPERTIES[1]

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It is the purpose of this paper to present the results of some single-valued measurements of the members of a group of soils, to compare the values obtained for the different kinds of measurements, and to discuss briefly the utility and probable significance of the determinations carried out.[2]

The chief object of making a "single-valued measurement" of a soil, as this term is now understood, is to obtain an expression which characterizes the physical behavior of the soil in some one or more ways, and which in addition may provide a useful and simple numerical value indicative of its texture. Several such measurements have been proposed and utilized from time to time, some of them with much success. In this country the hygroscopic coefficient and moisture equivalent have been used more extensively than any others. More recent single valued measurements which have been used here and elsewhere include the colloid content, the clay content, the shrinkage, the pore space, the sticky point, the stiffness, and the Atterberg values. The single-valued measurements which have been used in this work are as follows:

Colloid content by adsorption,
Content of clay finer than 2$\mu$ effective diameter,
Content of clay finer than 1$\mu$ effective diameter (determined by two different methods of pretreatment),
Air-dry moisture content,
Moisture equivalent,
Sticky point,
Rolling-out limit.

Fifty-seven soils were used. Detailed descriptions of 44 of these have already been given elsewhere (4). The same numbers have been retained for them, so that they may be identified readily with respect to grouping and origin. The 13 additional soils, numbered 51 to 54, 57, 59, and 61 to 67, comprise seven additional series from the San Joaquin Valley and adjacent foothills in California. They have been included for the purpose of increasing the geographical

[1] Paper read before the meeting of the Western Society of Soil Science at Corvallis, Oregon, June 17, 1930.

[2] The authors wish to thank Dr. Sylvia L. Parker for her criticism of the statistical portion of the paper.

distribution and broadening the already wide textural range. The entire group represents a wide variety in respect to age, origin, climate, and vegetation. All of the soils have been preserved in glass jars for at least 1 year, and in some cases for as long as 15 years, and were air-dry. Each was prepared before use by being passed through a 1-mm. screen, followed by thorough mixing.

## COLLOID BY ADSORPTION AND CLAY CONTENT

The influence of the colloid portion of the soil mass upon the physical, physico-chemical, and chemical properties of the whole is recognized. Various methods have been devised for measuring this part of the soil. Some involve indirect measurements in which the extent to which soils manifest certain properties characteristic of the colloid portion alone is measured. Others are more nearly direct, in that some arbitrary particle size limit is set as representing the approximate upper limit of size of the colloidal material, and separations or analyses based on these size limits are then used. The work of Anderson (3), Gile (7), and others has produced evidence favoring the use of $1\mu$ as the arbitrary upper limit of diameter for soil colloid, and according to Gile (8) this is the upper limit implied in the definition of soil colloid by the U. S. Department of Agriculture, Bureau of Chemistry and Soils. In a recent publication, however, Olmstead (11, p. 12) names the material below $2\mu$ effective diameter the "colloid" fraction. Colloid chemists usually consider a much smaller diameter as representing the upper limit of the colloidal state.

One indirect and two direct methods of measurement were used in the present case. W. O. Robinson's (13) procedure was followed in the determination of the water vapor adsorbed per gram of soil. The soils, previously sifted through a 1-mm. brass sieve, were exposed over 3.3 per cent sulfuric acid at 30°C. in a partially evacuated glass desiccator, stored for 5 days in a dark air thermostat. By dividing the water vapor adsorbed per gram of soil by 0.3 (13, 4) the percentage of colloid by adsorption was calculated.[3] The pipette method, following pretreatment with hydrogen peroxide and hydrochloric acid, washing, and shaking with dilute ammonia, was also used to measure the amounts of dispersible material having as upper limits of diameter $1\mu$ and $2\mu$ respectively. This pretreatment is part of the international method as adopted by the International Society of Soil Science (5). It aims at complete dispersion. A modified form of the aforementioned method, very similar to that adopted by the International Society of Soil Science for technical and petrographical purposes and which includes dispersion by shaking with dilute ammonia water only followed by pipette sampling, is a method that has been used to some extent as a rapid means of estimating the readily dispersible clay content of soil samples. This was used as a third method in the present series of measurements.

[3] The measurements of colloid by adsorption were made in connection with (4) by M. Tamachi.

Where both the 2 and 1$\mu$ samples were taken from the same suspension following the peroxide and acid pretreatment, the method followed was to sample for the material finer than 2$\mu$ first, the pipette being carefully lowered into, and withdrawn from the suspension. The cylinder was then left standing for the additional length of time necessary to allow for the settling of particles 1$\mu$ in diameter before again sampling with the pipette. In the international grouping of particle sizes, an effective diameter of 2$\mu$ is the upper limit of clay.

A comparison of the results obtained by these three methods, and a consideration of the yield of clay having a maximum particle diameter of 2$\mu$, are of interest. The results are given in table 1 for 44 soils. In 34 of the 44 soils, or in 77 per cent of the total, the highest quantity of colloid is calculated to be present when the water-vapor adsorption method is used, and comparisons are made with the results of the pipette methods of analysis, in which latter the quantities of material of 1$\mu$ in diameter and smaller are taken as measures of the colloid content. Furthermore, in 31 of the 44 soils, or in 70 per cent of the total, the colloid by adsorption exceeds the yield of material finer than 2$\mu$ obtained by the international method. In all cases but one (soil 6) the lowest yield of colloid is obtained when dispersion is produced by shaking with ammonia water only. Somewhat similar observations have been made by Gile (7) and by Joseph (9).

The results do not simplify the problem of the determination of the quantity of colloid in soils. They do indicate, however, on the one hand that pipette sedimentation determinations following the international method may provide misleading information through failing to take into account the possible presence of colloid coatings still adhering to the surfaces of the larger particles, or of micropores within the particles. Such particles might contribute considerably to the adsorptive capacity of the soil, without undergoing much reduction in settling velocity on account of lowered density. On the other hand the presence of soluble salts in the soil, and the nature of the replaceable bases (16) may slightly increase the quantity of adsorbed water vapor, and consequently the amount of colloid when calculated by the adsorption method. It is planned to study shortly the effect of the former possibility. It is clear that pretreatment by shaking with dilute ammonia water consistently gives figures considerably below those representing either the total dispersible colloid or the total quantity of water vapor-adsorptive material. Such incomplete dispersion obtained by simple shaking in ammonia water is of common occurrence. It has been explained by Wiegner (19).

Upon comparison of the quantities of material yielded by the international method of pretreatment and analysis, and having as upper limit of diameter 1 and 2$\mu$ respectively, it is found that for the 44 soils whose content of clay finer than 2$\mu$ ranged from 16 to 60 per cent, the average yield of clay of 1$\mu$ diameter and less, amounted to 89 per cent of the 2$\mu$ material for each soil. A range of from 36 to 100 per cent was obtained for this relationship. However, the lower values are rare, as only 4 of the 44 soils yielded an amount of material

TABLE 1

*Colloid and clay content as obtained by different methods of analysis, and air-dry moisture content, of 44 soils*

| SOIL NUMBER | COLLOID BY ADSORPTION | CLAY 1* | CLAY 2† | CLAY 3‡ | RATIO: CLAY 1 / CLAY 2 | AIR DRY MOISTURE CONTENT |
|---|---|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* | | *per cent* |
| 41 | 13.8 | 15.7 | 18.6 | 10.9 | 0.844 | 1.0 |
| 42 | 18.5 | 24.4 | 26.6 | 10.2 | 0.917 | 2.1 |
| 24 | 19.8 | 17.2 | 18.1 | 9.7 | 0.949 | 2.3 |
| 44 | 21.7 | 25.4 | 27.6 | 9.0 | 0.920 | 2.0 |
| 45 | 21.7 | 21.1 | 25.0 | 13.9 | 0.844 | 1.9 |
| 43 | 24.0 | 22.1 | 23.5 | 13.4 | 0.940 | 2.6 |
| 40 | 27.2 | 23.4 | 25.5 | 13.5 | 0.917 | 4.0 |
| 6 | 28.7 | 25.2 | 25.4 | 25.6 | 0.992 | 6.2 |
| 10 | 30.8 | 13.5 | 16.6 | 6.0 | 0.813 | 4.7 |
| 18 | 31.9 | 15.2 | 23.8 | 13.2 | 0.638 | 3.7 |
| 25 | 32.7 | 25.8 | 29.8 | 23.5 | 0.866 | 3.6 |
| 33 | 33.4 | 14.7 | 20.0 | 8.6 | 0.735 | 4.7 |
| 49 | 35.1 | 23.5 | 28.6 | 7.5 | 0.821 | 4.3 |
| 20 | 36.8 | 27.6 | 32.0 | 17.4 | 0.862 | 3.1 |
| 50 | 36.8 | 45.8 | 45.4 | 9.3 | 1.008 | 5.5 |
| 7 | 38.0 | 53.5 | 56.0 | 29.0 | 0.955 | 11.5 |
| 5 | 38.8 | 31.4 | 32.6 | 12.3 | 0.975 | 5.4 |
| 11 | 40.9 | 30.2 | 32.0 | 13.4 | 0.943 | 6.1 |
| 22 | 41.9 | 18.7 | 21.8 | 8.8 | 0.857 | 6.4 |
| 16 | 43.6 | 33.9 | 36.5 | 14.1 | 0.928 | 5.6 |
| 26 | 45.3 | 46.0 | 48.0 | 26.6 | 0.958 | 6.0 |
| 12 | 45.6 | 45.4 | 46.3 | 24.3 | 0.981 | 6.8 |
| 32 | 48.8 | 50.1 | 53.3 | 28.1 | 0.940 | 6.9 |
| 48 | 49.4 | 15.7 | 36.8 | 14.1 | 0.426 | 6.0 |
| 47 | 49.6 | 13.4 | 36.9 | 8.4 | 0.363 | 6.9 |
| 31 | 49.7 | 47.4 | 49.4 | 28.5 | 0.959 | 6.8 |
| 29 | 50.0 | 49.9 | 51.6 | 26.2 | 0.968 | 6.5 |
| 3 | 50.1 | 35.8 | 37.3 | 14.6 | 0.959 | 7.7 |
| 8 | 50.2 | 36.1 | 38.3 | 20.6 | 0.942 | 6.0 |
| 30 | 50.5 | 52.4 | 53.3 | 26.0 | 0.983 | 6.7 |
| 15 | 50.8 | 32.2 | 39.9 | 15.1 | 0.807 | 6.1 |
| 28 | 52.5 | 58.9 | 58.7 | 27.0 | 1.003 | 7.9 |
| 9 | 54.3 | 60.0 | 61.8 | 25.6 | 0.970 | 8.1 |
| 27 | 55.0 | 45.0 | 46.9 | 25.1 | 0.959 | 5.5 |
| 34 | 55.7 | 48.5 | 55.7 | 21.9 | 0.870 | 9.3 |
| 14 | 56.2 | 52.5 | 51.9 | 25.4 | 1.012 | 7.7 |
| 13 | 56.6 | 38.3 | 41.8 | 12.5 | 0.916 | 7.8 |
| 21 | 58.8 | 29.7 | 31.6 | 24.0 | 0.940 | 5.6 |
| 23 | 62.6 | 44.4 | 44.2 | 27.6 | 1.005 | 9.0 |
| 46 | 65.1 | 36.0 | 43.8 | 13.0 | 0.821 | 8.5 |
| 17 | 66.7 | 50.4 | 53.4 | 26.4 | 0.944 | 9.7 |
| 37 | 67.7 | 35.2 | 37.8 | 4.0 | 0.931 | 4.5 |
| 35 | 67.9 | 52.3 | 60.6 | 29.4 | 0.863 | 9.9 |
| 2 | 87.4 | 35.8 | 36.3 | 21.7 | 0.986 | 7.5 |

* Yield of material finer than 1$\mu$ effective diameter, following international method of pretreatment, and pipette method of analysis.

† Yield of material finer than 2$\mu$ effective diameter, following international method of pretreatment, and pipette method of analysis.

‡ Yield of material finer than 1$\mu$ effective diameter, following shaking over-night with dilute ammonia, and pipette method of analysis.

finer than 1$\mu$ equal to less than 80 per cent of the material finer than 2$\mu$, whereas 29 of the soils were found to contain quantities of material finer than 1$\mu$ amounting to 90 per cent or more of the material finer than 2$\mu$. The constancy of this ratio between 1 and 2$\mu$ material for soils containing different contents of clay is apparent from the values of the fourth and sixth columns of table 1.

## DETERMINATIONS OF THE STICKY POINT, THE ROLLING-OUT LIMIT,[4] AND THE NON-STICKY PLASTIC RANGE

The methods used for the determination of the air-dry moisture content and the moisture equivalent have been described in detail elsewhere (4). The sticky point was determined by placing about 10 gm. of air-dry soil on the hand and slowly adding water from a burette until the soil was wet and sticky. The wet soil was then kneaded in the hand by thumb and fingers until, through gradual drying, it reached such a state of moistness that it just failed to stick to the thumb when pressed. The pressure applied by the thumb should be approximately the same at each trial for stickiness. A moisture determination of the mass of kneaded soil was then made immediately by oven drying at 105°C., the moisture content on the dry basis being taken as a measure of the sticky point of the soil. This procedure is practically identical with that described by Keen and Coutts (10, p. 745). Five independent determinations of the sticky point were made by the same worker for each soil. The average range between the highest and the lowest result obtained in each set of five determinations was 1.92 per cent. The lowest such range was 0.5 per cent, and the highest 3.8 per cent. The results given are the averages of the five determinations. Keen and Coutts have pointed out (10, p. 758–760) the differences in results which may be expected between different workers making independent determinations upon the same soils.

The rolling-out limit was measured by using adaptations of Atterberg's (14) original method. For the more clayey soils the procedure was as follows: A rounded mass of about 3 gm. of soil, at approximately the sticky point, was placed on a glazed tile. The mass was then rolled with the flat side of a thin, light piece of wood, by applying a uniform pressure of such magnitude that when the board was moved parallel to the surface of the tile the soil was made to roll between board and tile, meanwhile assuming a cylindrical form which rolled out into a continuous thread. The thread was then destroyed and rolling recommenced. Repetitions of this treatment were continued until, as a result of gradual drying, the cylindrical thread which was formed, broke, upon rolling, into fragments 5 to 15 mm. long by about 2 to 3 mm. in diameter. The moisture content was then determined, and expressed as a measure of the rolling-out limit.

A slightly different method was found necessary for the determination of the rolling-out limit of the more sandy soils, because of the difficulty of rolling such

[4] Determinations of sticky point and rolling-out limit made by M. P. Baltazar.

soils into threads. Ten grams of soil were thoroughly wetted and moulded into a roughly cylindrical form. This cylinder was then placed on a glazed tile and very lightly rolled with the same wooden board as was used for the more clayey soils. During the rolling the surface of the tile was continually wiped dry to hasten the attainment of the end point. Finally, under such treatment, the soil was found to break into loose crumbs upon rolling. This was considered as the rolling-out limit, which was expressed numerically by making a moisture determination of the soil.

TABLE 2

*Results of five single-valued measurements for each of 20 soils*

| SOIL NUMBER | COLLOID BY ADSORPTION | CLAY* 2 MICRONS EFFECTIVE DIAMETER | MOISTURE EQUIVALENT | STICKY POINT | ROLLING-OUT LIMIT |
|---|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 19 | 21.4 | 23.1† | 27.3 | 29.6 | 27.7 |
| 44 | 21.7 | 27.6 | 20.2 | 20.0 | 13.5 |
| 45 | 21.7 | 25.0 | 18.6 | 17.2 | 12.4 |
| 43 | 24.0 | 23.5 | 20.1 | 17.4 | 15.2 |
| 40 | 27.2 | 25.5 | 23.7 | 24.3 | 15.5 |
| 10 | 30.8 | 16.6 | 26.3 | 26.0 | 25.1 |
| 25 | 32.7 | 29.8 | 32.2 | 21.6 | 13.8 |
| 33 | 33.4 | 20.0 | 27.0 | 24.5 | 21.7 |
| 50 | 36.8 | 45.4 | 27.3 | 30.1 | 17.1 |
| 5 | 38.8 | 32.6 | 29.1 | 33.2 | 22.9 |
| 36 | 40.5 | 35.0† | 29.6 | 32.5 | 19.4 |
| 16 | 43.6 | 36.5 | 32.6 | 36.4 | 25.7 |
| 12 | 45.6 | 46.3 | 29.8 | 30.4 | 18.7 |
| 38 | 48.2 | 39.9† | 29.2 | 31.7 | 18.1 |
| 31 | 49.7 | 49.4 | 30.6 | 33.4 | 17.9 |
| 29 | 50.0 | 51.6 | 34.8 | 33.9 | 24.8 |
| 3 | 50.1 | 37.3 | 33.7 | 37.7 | 29.4 |
| 8 | 50.2 | 38.3 | 37.7 | 36.4 | 22.6 |
| 27 | 55.0 | 46.9 | 28.9 | 29.1 | 15.5 |
| 21 | 58.8 | 31.6 | 38.6 | 40.8 | 30.1 |

* Following international method of pretreatment.

† Estimated values by means of the regression equation.

Five determinations, made by the same worker for each soil, gave an average range between highest and lowest results of 1.75 per cent for all soils. The lowest range for any set of five soils was 0.4 per cent, the highest 4.2 per cent. The results reported are the average of five separate determinations for each soil.

The non-sticky plastic range was determined by subtracting the rolling-out limit from the sticky point. There seems to be foundation for taking the sticky point as the upper limit of the plastic state, and hence for the measurement of the plastic range. As this may not yet meet with general agreement, and in order to avoid confusion, the term "non-sticky plastic range"

has been used. The word "plastic" strictly is not applied to an adhesive mass of soil, but is applicable for that region lying between the moisture content at which plasticity first becomes apparent (the rolling-out limit) and the

TABLE 3

*Results of three single-valued measurements and the ratios existing between some of these, for each of 34 soils*

| SOIL NUMBER | MOISTURE EQUIVALENT | STICKY POINT | STICKY POINT / MOISTURE EQUIVALENT | ROLLING-OUT LIMIT | ROLLING-OUT LIMIT / MOISTURE EQUIVALENT | NON-STICKY PLASTIC RANGE |
|---|---|---|---|---|---|---|
| | *per cent* | *per cent* | | *per cent* | | |
| 65 | 9.5 | 13.3 | 1.41 | 12.4 | 1.30 | 0.9 |
| 62 | 14.8 | 13.8 | 0.93 | 13.4 | 0.91 | 0.4 |
| 61 | 15.6 | 16.3 | 1.04 | 11.8 | 0.76 | 4.5 |
| 66 | 16.9 | 14.4 | 0.86 | 13.4 | 0.79 | 1.0 |
| 45 | 18.6 | 17.2 | 0.92 | 12.4 | 0.67 | 4.8 |
| 51 | 18.6 | 18.5 | 0.99 | 19.1 | 1.03 | −0.6 |
| 63 | 19.2 | 21.3 | 1.13 | 17.8 | 0.61 | 3.5 |
| 43 | 20.1 | 17.5 | 0.87 | 15.2 | 0.76 | 2.3 |
| 44 | 20.2 | 20.0 | 0.99 | 13.5 | 0.67 | 6.5 |
| 52 | 21.3 | 21.8 | 1.02 | 15.6 | 0.73 | 6.2 |
| 54 | 22.4 | 26.1 | 1.18 | 26.1 | 1.22 | 0.0 |
| 67 | 23.1 | 22.2 | 0.93 | 16.6 | 0.72 | 5.6 |
| 40 | 23.7 | 24.3 | 1.03 | 15.5 | 0.65 | 8.8 |
| 53 | 24.3 | 24.8 | 1.02 | 16.8 | 0.69 | 8.0 |
| 57 | 25.4 | 29.0 | 1.15 | 17.8 | 0.71 | 11.2 |
| 10 | 26.3 | 26.0 | 0.99 | 25.1 | 0.95 | 0.9 |
| 33 | 27.0 | 24.5 | 0.91 | 21.7 | 0.80 | 2.8 |
| 19 | 27.3 | 29.6 | 1.08 | 27.7 | 1.01 | 1.9 |
| 50 | 27.3 | 30.1 | 1.10 | 17.1 | 0.63 | 13.0 |
| 27 | 28.9 | 29.1 | 1.01 | 15.5 | 0.54 | 13.6 |
| 5 | 29.1 | 33.2 | 1.14 | 22.9 | 0.79 | 10.3 |
| 38 | 29.2 | 31.7 | 1.09 | 18.1 | 0.62 | 13.6 |
| 36 | 29.6 | 32.5 | 1.10 | 19.4 | 0.66 | 13.1 |
| 12 | 29.8 | 30.4 | 1.02 | 18.7 | 0.63 | 11.7 |
| 31 | 30.6 | 33.4 | 1.08 | 17.9 | 0.58 | 15.5 |
| 25 | 32.2 | 21.6 | 0.67 | 13.8 | 0.43 | 7.8 |
| 16 | 32.6 | 36.4 | 1.12 | 25.7 | 0.79 | 10.7 |
| 3 | 33.7 | 37.7 | 1.12 | 29.4 | 0.87 | 8.3 |
| 29 | 34.8 | 33.9 | 0.97 | 24.8 | 0.71 | 9.1 |
| 8 | 37.7 | 36.4 | 0.97 | 22.6 | 0.60 | 13.8 |
| 21 | 38.6 | 40.8 | 1.06 | 30.1 | 0.78 | 10.7 |
| 64 | 40.9 | 42.4 | 1.04 | 28.7 | 0.70 | 13.7 |
| 17 | 49.3 | 44.3 | 0.90 | 26.7 | 0.54 | 17.6 |
| 59 | 50.0 | 31.2 | 0.61 | 16.9 | 0.34 | 14.3 |
| Average... | .... | .... | 1.01 | .... | 0.74 | .... |

moistness at which the soil mass becomes adhesive (the sticky point). This range may or may not include the so-called "Fliessgrenze" of Atterberg.

The results of the determinations are contained in tables 2 and 3.

INTERRELATIONS OF THE SINGLE-VALUED EXPRESSIONS

The correlations existing between the colloid by adsorption and the air-dry moisture content, and the content of clay finer than $2\mu$ were examined for 44 soils. In addition the moisture equivalent, sticky point, and rolling-out limit were compared with the colloid by adsorption and with the content of clay finer than $2\mu$ for 20 of the soils, and their coefficients of correlation were determined. Finally, values of the correlation coefficient were obtained between the moisture equivalent and the sticky point, rolling-out limit, and the non-sticky plastic range, respectively, for 34 soils (6, 17, 18).

The results of these calculations are given in table 4, which gives for each correlation, in the last column, the probability that such a correlation would arise by random sampling of an uncorrelated population. The correlations are to be regarded as significant if the probability is low.

In 1916 Alway and Clark (1) suggested the utility of the determination of the hygroscopic moisture of soils as an index of their hygroscopic coefficient, and from numerous careful measurements of both values found that a simple ratio connected the two. They stated that, because of its simplicity, the hygroscopic moisture determination might well be considered as a means of measuring the hygroscopic coefficient wherever the samples were numerous and the labor involved in the direct determination considerable. In connection with their vapor pressure studies in 1925, Puri, Crowther, and Keen (12) pointed out the steepness of the vapor pressure-moisture content curves for soils in the general region of 50 per cent relative humidity, and stated that on that account the air-dry moisture content of a soil might be taken as well as any other single soil constant for characterizing the soil. Since then Keen and Coutts (10) have used the moisture content at 50 per cent relative humidity in that capacity. The high correlation, $r = 0.74 \pm .05$, obtained between measurements of the air-dry moisture content[5] and the colloid by adsorption for 44 soils is in general accord with conclusions to be drawn from the data of Alway and that of the Rothamsted workers.

An approximation to the percentage of colloid by adsorption may be obtained from the air-dry moisture content by means of the regression equation (18),

$$\bar{C} = M_c + r \cdot \frac{\sigma_c}{\sigma_w} (W - M_w)$$

where

$W$ = percentage of air-dry moisture content of particular soil for which an estimated value of colloid content is sought,

[5] No continuous record of the humidity of the air in the laboratory has been obtained. Nearly 500 readings of wet and dry bulb thermometers, kept in the laboratory at some distance from the sample storage room, have since been taken at intervals between 8 a.m. and 5 p.m. for the period September, 1930 to April, 1931. These gave an average relative humidity of 52 per cent.

$C$ = percentage colloid by adsorption,
$\bar{C}$ = estimated value of $C$,
$M_c$ = mean value of $C$,
$M_w$ = mean value of $W$,
$\sigma_c$ = standard deviation of $C$,
$\sigma_w$ = standard deviation of $W$,
and $r$ = correlation coefficient.

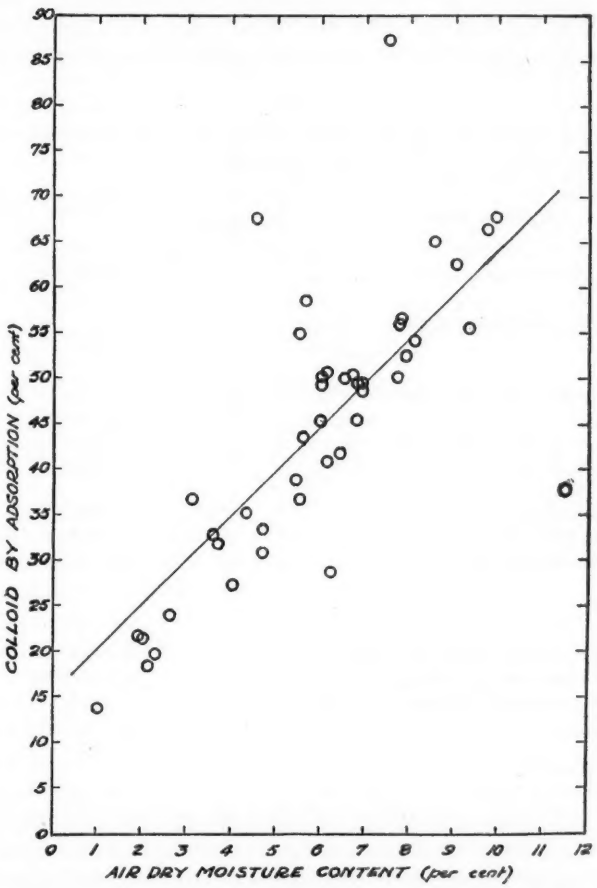


FIG. 1. RELATION BETWEEN AIR-DRY MOISTURE CONTENT AND COLLOID BY ADSORPTION

By calculation from the values of $W$ and $C$ contained in table 1 the regression equation is resolved into the expression, $\bar{C} = 4.91W + 15.6$. The closeness of agreement between estimated and experimental values of the colloid by adsorption is indicated by the scatter diagram and regression line of figure 1.

A somewhat lower degree of correlation is found to exist for these same soils between the colloid content and the content of clay finer than $2\mu$. This, together with the earlier observations concerning the relative magnitude of the two values, suggests that the measurement of particles having certain settling velocities does not provide a true measure of the colloidally active material.

The high correlations obtained between the colloid by adsorption, and the moisture equivalent and sticky point simply provide further confirmation of the general truth that the higher the colloid content of a soil the higher its water-holding capacity and the higher its moisture content before it becomes

TABLE 4

*Correlation coefficients between pairs of single-valued expressions, calculated from data of tables 1, 2, and 3*

| VALUES CORRELATED | NUMBER OF SAMPLES USED IN CORRELATION | CORRELATION COEFFICIENT ($r$) | PROBABILITY* (6) |
|---|---|---|---|
| Colloid by adsorption and clay $<2\mu$ | 44 | $0.65\pm0.06$ | $<0.01$ |
| Colloid by adsorption and air-dry moisture content | 44 | $0.74\pm0.05$ | $<0.01$ |
| Colloid by adsorption and moisture equivalent | 20 | $0.83\pm0.05$ | $<0.01$ |
| Colloid by adsorption and sticky point | 20 | $0.83\pm0.05$ | $<0.01$ |
| Colloid by adsorption and rolling-out limit | 20 | $0.42\pm0.12$ | $0.05<0.1$ |
| Clay $<2\mu$ and moisture equivalent | 20 | $0.50\pm0.11$ | $0.02<0.05$ |
| Clay $<2\mu$ and sticky point | 20 | $0.54\pm0.11$ | $0.01<0.02$ |
| Clay $<2\mu$ and rolling-out limit | 20 | $-0.01\pm0.15$ | High, no correlation |
| Moisture equivalent and sticky point | 34 | $0.89\pm0.02$ | $<0.01$ |
| Moisture equivalent and rolling-out limit | 34 | $0.61\pm0.07$ | $<0.01$ |
| Moisture equivalent and non-sticky plastic range | 34 | $0.76\pm0.05$ | $<0.01$ |

* See page 372.

sticky. The much less close association obtained between the clay finer than $2\mu$ and the moisture equivalent and sticky point indicates that the adsorptive surfaces are of relatively more significance in these particular values than the particle sizes.

From the correlation coefficients of table 4 it appears that the measurement of the colloid by water-vapor-adsorption is a much more significant criterion of the probable physical behavior of a soil than is a measure of its clay finer than $2\mu$ following the hydrogen peroxide-hydrochloric acid pretreatment.

The value of the rolling-out limit is very much less affected by the colloid content and by the clay finer than $2\mu$.

The values reported in table 3, and their corresponding correlations in table 4, are of much interest. Very close relationships exist between the three single-valued measurements reported, and also between the moisture equivalent and the non-sticky plastic range. These results indicate that a rapid field appraisal may be made of the moisture condition of a given soil in terms of its relative moistness for purposes of decisions concerning moisture supply and timeliness of performing tillage operations. The sticky point represents a higher point on the moisture scale than does the rolling-out limit. Two-thirds of the 34 soils examined had a sticky point within $\pm 10$ per cent of the moisture equivalent. Of the remainder, four soils had sticky points below 90 per cent of the moisture equivalent, ranging from 61 to 86 per cent of that value, and seven had sticky points above 110 per cent of the moisture equivalent, ranging from 112 to 141 per cent of it. The average ratio, sticky point: moisture equivalent, amounted to 1.01. In view of the significance of the moisture equivalent as an index of the moisture holding capacity of the medium textured soils, as shown by Alway and McDole (2), Shaw (15), and others, the rather close agreement with it of the sticky point, a readily determined quantity, is interesting and useful.

The significance of the rolling-out limit is that it represents the lower limit of the plastic consistency for low forces of compression. It is apparently a means of determining the lower moisture limit of easy puddling and of easy reduction of pore space. Over one-half of the 34 soils were found to have a rolling-out limit between 64 and 84 per cent of the moisture equivalent. Nine of the remaining soils had rolling-out limits below 64 per cent of the moisture equivalent and six had values above 84 per cent of it. The average ratio rolling-out limit: moisture equivalent was 0.74.

The close correlation between the non-sticky plastic range and the moisture equivalent indicates that as the texture of the soils becomes finer, as expressed by the moisture equivalent, a wider range is to be expected between the rolling-out limit and the sticky point. That is to say, the lower limit of easy puddling becomes farther and farther removed from the sticky point. By virtue of the relationship existing between the sticky point and the moisture equivalent this also means that for the heavier soils, in terms of absolute moistness, usually the rolling-out limit is farther from the moisture equivalent.

The relationships existing between moisture equivalent, sticky point, and rolling-out limit are shown graphically in figure 2, which is a combined scatter diagram of sticky points and rolling-out limits plotted against moisture equivalents. The non-sticky plastic range is indicated in each case by a vertical bar connecting the sticky point with the rolling-out limit for the same soil. The regression lines for the sticky point and rolling-out limit on the moisture equivalent, with their equations, have been drawn in the figure, assuming linear associations. The shaded portion between the two lines represents the non-

sticky plastic range, and the position which it may be expected to occupy for different values of the moisture equivalent. The divergent nature of the boundaries of this range as the soils increase in fineness of texture is clearly shown. The sticky point and rolling-out limit may be expected to exhibit a tendency to coincide in the vicinity of moisture equivalent = 9.8 per cent. It

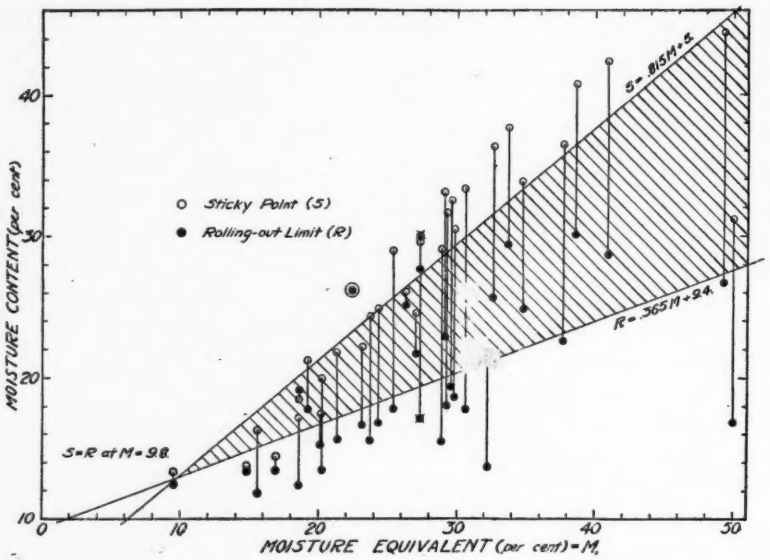


FIG. 2. RELATION BETWEEN MOISTURE EQUIVALENT, STICKY POINT, ROLLING-OUT LIMIT AND NON-STICKY PLASTIC RANGE

is somewhat doubtful whether the inversion of values to the left of $M = 9.8$ per cent would be consistently obtained experimentally, although inversion is known to occur (e.g. soil 51).[6]

## SUMMARY

Measurements of several single-valued soil properties; namely, colloid content by adsorption, clay finer than $2\mu$ and clay finer than $1\mu$ following treatment with hydrogen peroxide and hydrochloric acid, clay finer than $1\mu$ following shaking with ammonia water, air-dry moisture content, moisture equivalent, sticky point, and rolling-out limit, were made with a large variety of soils.

[6] Since preparation of this article there has come to our attention a paper by J. C. Russel, "Variations in the B Horizon" in Bul. 9, Rep. 8th Ann. Meeting American Soil Survey Association, pp. 100–112A, March, 1928. In it Russel indicated a relation between clay content and "plasticity number," the latter being the difference between the "Ausrollgrenze" and the "Fliessgrenze." This is in general qualitative agreement with our results.

A comparison of the results for the measurement of colloid by adsorption and clay fractions representing different upper size limits, showed that the adsorption method gave higher results on the whole than were obtained for a measure of the peroxide-hydrochloric acid dispersed clay finer than $2\mu$, and that the clay finer than $1\mu$ constituted on the average nearly 90 per cent of the total clay having $2\mu$ as the upper limit, for soils of varying clay content. The ammonia pretreatment yielded much lower quantities of clay finer than $1\mu$ than did the peroxide-hydrochloric acid pretreatment. The results indicate that the international method of dispersion and analysis does not permit complete expression of the probable colloid content of a soil, possibly because of indispersible colloid shells at the particle surfaces, and because of the presence of micropores, neither of which is taken into account by a mere expression of size distribution.

Significantly high correlations were found to exist between the moisture equivalent and the sticky point, colloid and moisture equivalent, colloid and sticky point, moisture equivalent and non-sticky plastic range, colloid and air-dry moisture content, colloid and clay finer than $2\mu$, and moisture equivalent and rolling-out limit, these being here arranged in descending order of their correlation coefficients. Correlations of a lower order were obtained for clay and sticky point, clay and moisture equivalent, and colloid and rolling-out limit. No significant correlation was found between clay and rolling-out limit.

The utility of the relationship between the laboratory air-dry moisture content and the colloid content by adsorption is pointed out.

It is suggested that the sticky point and the rolling-out limit may have considerable significance in the rapid appraisal of soil moisture conditions in the field. A coincidence, or even an inversion, in the values of the sticky point and the rolling-out limit, for which the sticky point is normally the higher, may be expected to occur with soils having amoist ure equivalent in the neighborhood of 9.8 per cent.

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# PHOTOSENSITIZED OXIDATION OF AMMONIA AND AMMONIUM SALTS AND THE PROBLEM OF NITRIFICATION IN SOILS

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Most plants get the nitrogen they require from the soil, where it is present in the form of nitrates, ammonium salts, or complex nitrogenous compounds. Ammonium salts and other nitrogenous compounds must first be oxidized to nitrites and nitrates before they can be utilized by the plant. It has long been known that ammonia and ammonium salts are oxidized in the soil to nitrites and nitrates. It is now universally believed that the nitrification in soils is due entirely to the action of bacteria. The biological character of the nitrification process was first indicated by Müller (12) and by Soyka (14). From the painstaking researches of Warington, Frankland, and Winogradsky, we now know that this nitrification is really due to the joint action of two organisms, one of which converts ammonium salts to nitrites and the other of which brings about the oxidation of nitrites to nitrates but has no effect on ammonium salts.

From our experiments on the photosensitized oxidation of ammonia and ammonium salts in the presence of sunlight and various photosensitizers, we are confident that the nitrification in the soil is, at least in part, photochemical in nature, taking place at the surface of various photosensitizers under the influence of sunlight. In the absence of sensitizers there is a noticeable, though very slight, oxidation [compare Dhar and Sanyal (4)]. It has been noted that the same reaction takes place in ultra violet light (3). Under the action of sunlight and in the presence of sensitizers, there is vigorous oxidation of ammonia and ammonium salts to nitrite.

## EXPERIMENTAL

Titania, zinc oxide, cadmium oxide, sodium uranate, alumina, and silica were used as photosensitizers. They were prepared by precipitation from the aqueous solutions of the corresponding pure salts and were usually dried, ignited, and finely powdered, for freshly precipitated substances, as a rule, are less active than the ignited materials.

Solutions of ammonia or ammonium salts were exposed to sunlight, with small quantities of the photosensitizer, and a current of air free from nitrous fumes was passed through the solutions, so that the solid photocatalysts were kept in suspension. Glass beakers were generally employed; and as glass transmits only up to 3,500 Å it may be concluded that radiations of shorter

wave length do not take part in our reactions. In all the cases studied, there was no dark reaction.

Nitrite was tested for qualitatively, by the Griess reagent and starch potassium iodide; it was estimated quantitatively, by the usual permanganate method.

### *Influence of anions*

To study the influence of anions, 100 cc. of 0.2 *N* solutions of aqueous ammonia, ammonium carbonate, ammonium phosphate, ammonium chloride, and ammonium sulfate solutions were taken in separate pyrex glass beakers, to each of which was added 1 gm. of Merck's zinc oxide. The solutions were then exposed to the sun for three hours, while a current of air was passed through at an approximately uniform rate. The beakers were kept covered with glass plates. The resulting nitrite was estimated by the permanganate method, which necessitated the use of manganous sulfate whenever chloride was present. The results are shown in table 1.

TABLE 1

*Influence of anions on nitrite formation*

100 cc. of 0.2 *N* salt solution. Time of exposure to the sun, 3 hours

| SALT SOLUTION USED | VOLUME OF 0.025 *N* $KMnO_4$ SOLUTION CORRESPONDING TO THE AMOUNT OF NITRITE FORMED |
|---|---|
| | *cc.* |
| $NH_4OH$ | 7.1 |
| $(NH_4)_2CO_3$ | 3.0 |
| $(NH_4)_2HPO_4$ | 2.0 |
| $(NH_4)_2SO_4$ | 2.4 |
| $NH_4Cl$ | 2.0 |

The results shown in table 1 indicate that anions do not have much influence on the rate of oxidation; the pH of the solution, however, seems to have a great influence. The pH values, therefore, of 0.2 *N* aqueous solutions of the salts listed in table 1 were determined.

| *Salt solution* | *pH* |
|---|---|
| $NH_4OH$ | Above 10 |
| $(NH_4)_2CO_3$ | 8.1 |
| $(NH_4)_2SO_4$ | 6.9 |
| $(NH_4)_2HPO_4$ | 8.0 |
| $(NH_4)Cl$ | 6.9 |

On the whole, acidity seems to decrease the rate of oxidation and basicity to increase it; though an exception to this rule is noticed in the case of ammonium phosphate, which requires further investigation.

*Comparative experiments in glass and quartz vessels*

The rate of oxidation is greater in a quartz vessel than in a glass one. The nitrite formed from 100 cc. of 0.2 *N* aqueous ammonia exposed in a *glass* beaker for three hours corresponds to 7.1 cc. of 0.025 *N* $KMnO_4$, whereas the nitrite formed from the same volume of $NH_4OH$ exposed in a *quartz* beaker for the same length of time, both containing 1 gm. of ZnO, required 11 cc. of the same $KMnO_4$. This fact can be easily explained as follows: Zinc oxide shows marked absorption in the blue, violet, and ultra-violet regions of the spectrum. In sunlight, at least in tropical countries, the ultra-violet region extends to 2,900 Å. When the experiment is carried on in a glass vessel only radiation up to 3,500 Å can be utilized, because glass does not transmit beyond this region, whereas in a quartz vessel all the radiations in the solar spectrum up to 2,900 Å can be utilized. Evidently more of the solar energy is utilized in a quartz vessel than in a glass vessel; and hence the greater rapidity of the reaction.

The accumulation of nitrite seems to have little or no influence on the rate of oxidation of ammonia.

*Comparative activities of the various photosensitizers*

Semi-quantitative experiments, the results of which are not recorded here, indicate the following order for the photosensitizing activity of the various substances:

$$TiO_2 > ZnO, CdO > (Na_2U_2O_7) > Al_2O_3 > MgO, SiO_2$$

we hope to be able to elucidate the varying activities of these substances from a study of their absorption spectra.

*Mechanism of the oxidation*

According to Winther (14) oxygen can be ozonized in the presence of zinc oxide by irradiation with light of wave lengths which have no action on oxygen alone. We have found that, when a suspension of zinc oxide in water is exposed to sunlight, ozone and hydrogen peroxide are formed. It is known that ozone interacts with ammonia or ammonium salts according to the equation

$$NH_4OH + O_3 = HNO_2 + 2\,H_2O$$

and the nitrous acid interacts with ammonia giving ammonium nitrite

$$HNO_2 + NH_4OH = (NH_4)NO_2 + H_2O$$

## DISCUSSION

These experiments on the photochemical oxidation of ammonia and ammonium compounds to nitrite are remarkably interesting from the point of view of the nitrification in soils. Hitherto it has been believed that the nitrifi-

cation is entirely due to bacteria. We are now convinced that it is, at least in part, photochemical in nature, taking place at the surface of various soil photocatalysts, which absorb the solar radiations. There are some important facts of the nitrification process which do not fit in with the bacterial hypothesis. The present hypothesis not only accounts for these facts, unexplainable by the older hypothesis, but it is also in line with other facts of the nitrification in soils.

The following are some of the important facts unexplainable on the bacterial hypothesis

Omeliansky (13) and Meyerhof (11) have shown that for the bacterial nitrification process a high concentration of ammonia or nitrite is harmful—the optimum concentration is about 0.05 per cent with a second optimum at 0.1 per cent. More than 0.3 per cent interferes with the process. In the so-called niter-spots, a high concentration of nitrite and nitrate, as much as 5 per cent of the bulk of the soil, has been noticed. The biological explanation cannot be entirely sufficient for the accumulation of nitrites and nitrates, for the high concentration of nitrite and nitrate formed should preclude the action of nitrifying organisms in the later stages.

The researches of Traaen (15) McBeth and Smith (10) and Lyon, et al. (8) have shown that the nature of the soil also influences the nitrification.

A periodic variation of the nitrite and nitrate content of soils has been noticed by several workers [Compare Batham, (1) ]. The nitrite and nitrate content of soils rises to a maximum in the summer and falls to a minimum in the winter. These results are explained by some as being due to the activating influence of the sun on the nitrifying bacteria. How far this is true cannot be said with certainty at present, but our photochemical hypothesis offers a ready explanation of this phenomenon.

The preceding facts can be explained as follows:

We have shown that the oxidation of ammonium compounds to nitrite takes place at the surface of various oxides, e.g. titania, zinc oxide, cadmium oxide, alumina, and silica in the presence of sunlight. We have also shown that the accumulated nitrite has no influence on the rate of oxidation of ammonium compounds. In the soil, alumina and silica and also titania, undoubtedly occur. The photochemical hypothesis thus sets no limit to the amount of nitrite that can be formed. As such, the accumulation of nitrites and nitrates in large amounts in the nitrite spots is explained.

The influence of soils and the varying activity of different soils also find a ready explanation by this hypothesis. All soils contain alumina and silica; all soils are therefore more or less active in the nitrification process. It has been found that titanium, zinc, and cadmium oxides are very efficient photocatalysts for the nitrification process and are far more active than alumina and silica. Therefore, if the soil contains in addition to alumina and silica, titania or zinc and cadmium oxides or some other efficient photocatalyst as yet undiscovered, it will be very active in the nitrification process and hence very fertile. In consonance with this presumption, it has been recorded by Geilman (5) that most fertile soils contain titania in quantities as large as 0.3 to 0.6 per cent. It also occurs in the ashes of all plants up to 0.27 per cent. According to the same worker, the fertility of the soil depends to some extent on the titanium content of the soil.

The observations made in the present investigation that basicity favors the photochemical oxidation and that acidity is harmful are in agreement with similar observations on the nitrification process in the soil made by various workers [Compare Lyon and Bizzel (8)].

Besides the evidence so far adduced in favour of the photochemical hypothesis, the following also may be mentioned. St. von Bazarewsky (2), Koch (7), and Lyon and his coworkers (9) have found that *the nitrification process is most active near the surface of the soil.* This observation forms a necessary deduction from our photochemical hypothesis, for most the of light falling is absorbed by the outermost layers of the soil and little is allowed to pass through. As light is essential for the photochemical nitrification, it is but natural that there is the greatest oxidation where there is the maximum absorption of radiant energy.

## SUMMARY

Oxidation of ammonia and its compounds in solution to nitrite has been effected by sunlight in the presence of photosensitizers. The velocity of oxidation is greater in quartz vessels than in glass vessels.

The various photosensitizers fall into the following order with respect to their activity:

$$TiO_2 > ZnO, CdO > Na_2U_2O_7 > Al_2O_3 > SiO_2$$

Basicity favors the oxidation, and acidity decreases the velocity of oxidation.

On the basis of the foregoing experiments, a new mechanism for the nitrification in soils has been suggested. We believe that the nitrification in soils is, at least in part, photochemical in nature, taking place at the surface of various photocatalysts present in the soil under the influence of sunlight.

The bacterial explanation of the soil nitrification process has been shown to be inadequate. Facts which do not fit in with this hypothesis, have been shown to be explicable on the photochemical hypothesis. We believe that the bacterial and photochemical processes take place side by side.

Further work is in progress in this line.

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# CHANGES PRODUCED IN NITROGENOUS COMPOUNDS BY RHIZOBIUM MELILOTI AND RHIZOBIUM JAPONICUM[1]

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During the past few years there have been many investigations of the physiological activities of the legume bacteria in the attempt to gain a better understanding of their species relationships and nitrogen fixing activities. Although much has been learned of the physiology of these organisms there are still many phases of their activity which need further study.

Changes in the nitrogenous compounds in media in which the legume bacteria have been grown have been noted by certain investigators. Hills (5) and Allison (1) noted the disappearance of nitrates in solutions inoculated with certain species of Rhizobium. Lewis and Nicholson (7) found a utilization of nitrates, accompanied by the production of nitrites by certain strains of *Rhizobium meliloti* and *Rhizobium japonicum*. The production of nitrites was also noted by Leonard (6).

Many investigators found a more vigorous growth in media containing combined nitrogen, but did not determine to what extent the utilization of nitrogen was responsible for the increased growth. Müller and Stapp (8) found that potassium nitrate, sodium nitrate, ammonium chloride, ammonium sulfate, ammonium nitrate, asparagine, aspartic acid, and uric acid served as suitable sources of nitrogen for the legume bacteria. Urea was found to be somewhat suitable as a nitrogen source, alanine and leucine were suitable for certain groups, and glycocoll and hippuric acid did not serve as suitable nitrogen sources for the legume bacteria. These authors also noted that the legume bacteria produced a proteolytic enzyme having a weak action. The presence of an oxidizing enzyme having a weak action was noted by Fred (4) but he was unable to demonstrate either invertase or proteolytic reducing enzymes.

The recent work of Baldwin, Fred, and Hastings (2) suggested that there may be a relationship between the various species of the legume bacteria and the proteins present in the seeds of leguminous plants. It seems then, that a study

[1] Part of a thesis submitted in partial fulfillment of the requirements of the degree of doctor of philosophy at the Iowa State College.

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of the changes brought about by the legume bacteria in media containing nitrogen may have some significance. The results of investigation along this line are reported here.

## EXPERIMENTAL

In the first experiments an attempt was made to determine whether there was a difference in the nitrogen changes produced by *Rhizobium meliloti* and *Rhizobium japonicum*. Since no nitrogen was fixed in earlier experiments, compounds containing nitrogen were added in all of these tests. The basic medium consisted of

| | |
|---|---|
| Mannitol | 10.0 gm. |
| $K_2HPO_4$ | 0.5 gm. |
| $MgSO_4$ | 0.2 gm. |
| NaCl | 0.2 gm. |
| $CaCO_3$ | 3.0 gm. |
| $FeCl_3$ | Trace |
| $MnSO_4$ | Trace |
| Distilled water | 1,000 cc. |

The compounds tested were glycocoll, asparagine, tyrosine, peptone, and urea. With the exception of peptone and urea these were added at the rate of 0.5 gm. to a liter of solution. Urea was added at the rate of 2 gm. a liter and peptone at the rate of 1 gm. a liter. After the reaction of the media had been adjusted to pH 7.0, 100-cc. portions were placed in 500-cc. Erlenmeyer flasks, sterilized, and duplicate flasks inoculated as follows —

107. *Rhizobium meliloti*
112. *Rhizobium meliloti*
406. *Rhizobium japonicum*
407. *Rhizobium japonicum*

These organisms had previously been tested for ability to produce nodules on the host plant and for purity in milk and on potato slants. After three weeks incubation at room temperature the solutions were analyzed for total nitrogen, ammonia, nitrates, nitrites, and amino acid nitrogen. The methods used were as follows:

*Total nitrogen.* The determination of total nitrogen was made on a 25-cc. aliquot from each of the solutions. A modified Kjeldahl method was used in which a salt mixture containing sodium sulfate, copper sulfate, and ferrous sulfate was added to hasten digestion. In the solutions which contained nitrates or nitrites in measurable amounts the method of Davisson and Parsons (3) was used.

*Ammonia.* An aeration method was used for the determination of ammonia. Twenty-five cubic centimeters of the sample was placed in a large test tube, 0.5 gm. of sodium carbonate added, and the solution aerated. The ammonia was collected in 0.1 *N* sulfuric acid and titrated.

*Nitrites.* The test for nitrites was made according to the sulfanilic acid-alpha-naphthylamine method.

*Nitrates.* Nitrates were determined on the same sample used for the nitrite determination. A small amount of zinc dust was added to reduce the nitrates to nitrites, which could then be detected by the aforementioned reagents.

*Amino acids.* The Sorenson formol titration method was used for the determination of amino acids. For this determination, 25 cc. of the sample was taken. The amino acid utilized was obtained by subtracting the sum of the ammonia produced and the amino acid remaining from the amino nitrogen present in the check solutions.

These determinations were carried out as rapidly as possible in order to prevent any change due to contamination after the solutions had been exposed to the laboratory air.

The results obtained in the study of the changes produced in glycocoll appear in table 1. Both strains of *Rhizobium meliloti* utilized this amino acid. Part of the loss of amino acid nitrogen can be accounted for by the ammonia produced, but some of it was apparently utilized by these organisms in the

TABLE 1

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 0.5 gm. of glycocoll to a liter*

(Results expressed in milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | AMINO ACID TOTAL | AMINO ACID UTILIZED | TOTAL NITROGEN |
|---|---|---|---|---|
| Check | 0.28 | 9.24 | None | 8.68 |
| Check | 0.56 | 8.96 | None | 8.96 |
| 107 | 4.48 | 3.36 | 1.68 | 9.52 |
| 107 | 4.20 | 2.80 | 2.52 | 9.52 |
| 112 | 6.16 | 0.56 | 2.80 | 9.80 |
| 112 | 6.16 | 0.84 | 2.52 | 8.96 |
| 406 | 0.56 | 8.68 | 0.28 | 9.80 |
| 406 | 0.28 | 9.24 | −0.14 | 9.52 |
| 407 | 0.28 | 9.24 | −0.14 | 8.96 |
| 407 | 0.28 | 9.24 | −0.14 | 9.52 |

building up of their protein. The solutions inoculated with the two strains of *Rhizobium japonicum* did not show any evidence of growth during the period. Apparently the glycocoll was toxic to them in this concentration.

The variations in total nitrogen were within the limit of experimental error. They do show, however, that no appreciable amounts of nitrogen were gained or lost from the solutions during the period of incubation. Qualitative tests showed only traces of nitrites in the check solutions and in the solutions inoculated with organisms 112, 406, and 407. The small amount of nitrite present had apparently been utilized by organism 107. No nitrates were found in any of the solutions.

The changes in urea are shown in table 2. Some ammonia was produced by all of the organisms tested, but there was no significant difference between the two species. The loss of nitrogen found in some of the solutions was probably due to a loss of ammonia, as all of the organisms tested made this medium more alkaline. Nitrites and nitrates were absent from all of the cultures. No amino acid nitrogen could be detected in any of the solutions.

Table 3 shows the changes produced in asparagine. The production of ammonia and the decrease in amino acid nitrogen were much greater by the one strain of soybean bacteria than by the alfalfa bacteria. The Sorenson formol titration method was apparently not well suited for the determination of amino acid nitrogen in asparagine, but the results were at least comparative. In as-

TABLE 2

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 2.0 gm. of urea to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | AMINO ACID TOTAL | TOTAL NITROGEN |
|---|---|---|---|
| Check | 6.16 | None | 95.8 |
| Check | 5.88 | None | 95.5 |
| 107 | 9.80 | None | 89.1 |
| 107 | 9.80 | None | 88.5 |
| 112 | 17.91 | None | 96.4 |
| 112 | 17.63 | None | 94.0 |
| 406 | 9.80 | None | 90.5 |
| 406 | 9.80 | None | 89.8 |
| 407 | 11.20 | None | 90.5 |
| 407 | 10.92 | None | 90.5 |

TABLE 3

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 0.5 gm. of asparagine to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | AMINO ACID TOTAL | AMINO ACID CHANGED* | TOTAL NITROGEN |
|---|---|---|---|---|
| Check | Trace | 4.76 | 0 | 11.20 |
| Check | Trace | 4.76 | 0 | 10.58 |
| 107 | 2.52 | 3.08 | 1.68 | 11.06 |
| 107 | 3.08 | 2.52 | 2.24 | 10.92 |
| 112 | 2.52 | 3.08 | 1.68 | 11.34 |
| 112† | .... | .... | .... | ..... |
| 406† | .... | .... | .... | ..... |
| 406† | .... | .... | .... | ..... |
| 407 | 6.16 | 0.00 | 4.76 | 10.36 |
| 407 | 6.16 | 0.00 | 4.76 | 10.36 |

* Represents difference between total amino acid in check and inoculated solutions.

† Culture contaminated.

paragine one-half of the nitrogen is present in an amino group and the other half in an amido group. The determination did not show which of these groups was changed, but apparently both were acted upon by *Rhizobium japonicum* since the amount of nitrogen changed to ammonia was greater than the amount of nitrogen present as either amino or amido nitrogen. It is probable

also that the two strains of *Rhizobium meliloti* changed some of the nitrogen present in both groups. There was no significant change in the total nitrogen content of any of the solutions, and no nitrates or nitrites were produced in this medium.

The changes produced in tyrosine are shown in table 4. Although there appeared to be a slight utilization of this amino acid there was no apparent differ-

TABLE 4

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 0.5 gm. of l-tyrosine to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | AMINO ACID TOTAL | AMINO ACID UTILIZED | TOTAL NITROGEN |
|---|---|---|---|---|
| Check | None | 2.80 | None | |
| Check | None | 2.80 | None | |
| 107 | None | 2.22 | 0.58 | |
| 107 | None | 1.68 | 1.12 | |
| 112 | None | 1.96 | 0.84 | Not determined |
| 112 | None | 1.96 | 0.84 | |
| 406 | None | 1.96 | 0.84 | |
| 406 | None | 1.96 | 0.84 | |
| 407 | None | 1.96 | 0.84 | |
| 407 | None | 1.96 | 0.84 | |

TABLE 5

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of peptone to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | AMINO ACID TOTAL | AMINO ACID UTILIZED | TOTAL NITROGEN |
|---|---|---|---|---|
| Check | None | 0.84 | None | 7.28 |
| Check | None | 0.84 | None | 7.28 |
| 107 | None | 0.42 | 0.42 | 7.84 |
| 107 | None | 0.56 | 0.28 | 7.84 |
| 112 | None | 0.28 | 0.56 | 7.28 |
| 112* | ..... | .... | .... | .... |
| 406 | None | Lost | Lost | 7.84 |
| 406 | None | 0.56 | 0.28 | 7.84 |
| 407 | None | 0.56 | 0.28 | 7.56 |
| 407 | None | 0.56 | 0.28 | 7.84 |

* Culture contaminated.

ence in the ability of the two species to utilize tyrosine. No ammonia, nitrates or nitrites could be detected in any of the solutions. An interesting difference in the color of the solutions was noted. The check solutions were clear and colorless, those inoculated with the organisms 107 and 112 were golden brown, and those inoculated with 406 and 407 were a very dark brown. An explanation of the differences in colors produced is given later.

Table 5 shows the nitrogen changes which were brought about in a peptone solution. A small amount of amino acid nitrogen was measured by the formol titration method, part of which was utilized by the organisms tested. No ammonia, nitrates, or nitrites were produced in the medium. There was no appreciable increase or decrease in the total nitrogen content of the solutions.

In view of the differences found in the changes in the nitrogenous compounds tested in the foregoing experiments it was decided to carry out further tests, using different strains of the same two species of bacteria, and including a study of still other nitrogenous compounds. The organisms used were the following:

| | | |
|---|---|---|
| 113. | *Rhizobium meliloti* | |
| 130. | *Rhizobium meliloti* | Type A strain |
| 131. | *Rhizobium meliloti* | Type B strain |
| 132. | *Rhizobium meliloti* | Type B strain |
| 133. | *Rhizobium meliloti* | |
| 413. | *Rhizobium japonicum* | Type A strain |
| 414. | *Rhizobium japonicum* | Type B strain |
| 415. | *Rhizobium japonicum* | Type B strain |
| 416. | *Rhizobium japonicum* | Type A strain |

The four strains of *Rhizobium meliloti* (strains 130, 131, 132, and 133) and the four strains of *Rhizobium japonicum* (strains 413, 414, 415, and 416)[2] had previously been divided into two types as shown by Stevens (9) and Wright (11). The type A strains were rapid growers and fixed larger amounts of nitrogen in the host plant than the type B strains. Organism 113 was chosen because it produced a very marked increase in acidity when grown in yeast mannitol solution. Organism 133, although not classified by Stevens, appeared to be more like the type A strain 130 than the type B strains.

The organisms were tested for ability to inoculate the host plant and for purity on milk.

The nitrogenous compounds used in these tests included some aliphatic amino acids, some aromatic amino acids; some compounds having an amido group, such as asparagine and urea; and two inorganic nitrogenous compounds, ammonium sulfate and potassium nitrate.

The basic medium used was the same as in the previous tests, but the amount of the nitrogenous compound added was increased to 1.0 gm. to each liter. The incubation period was increased to five weeks. At the end of the incubation period yeast mannitol agar slants were inoculated with a suspension from each flask, and the type of growth was noted to verify the purity of the cultures at the end of the experiment. The species of Rhizobium tested have a characteristic type of growth on yeast mannitol agar slants which helps to differentiate them from other organisms.

The methods of analyses were the same as were used in the previous tests, except that the micro Van Slyke method (10) was used for the determination of

[2] Obtained through the courtesy of Dr. I. L. Baldwin of the Wisconsin Agricultural Experiment Station.

amino acid nitrogen. This was slightly modified by substituting the large deaminizing bulb from the macro apparatus, thus permitting the use of a 10-cc. aliquot instead of the 2-cc. aliquot used in the micro method. By this change more accurate determinations were made possible. The determination of nitrates and nitrites in the solutions receiving additions of potassium nitrate was made by reducing the nitrates and nitrites to ammonia by means of Devarda's alloy and distilling the ammonia produced into standard acid.

The changes produced in the glycocoll solution by the organisms tested are shown in table 6. The concentration of glycocoll used was apparently above

TABLE 6

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of glycocoll to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | AMINO ACID TOTAL | AMINO ACID UTILIZED | TOTAL NITROGEN |
|---|---|---|---|---|
| Check | None | 21.18 | None | 20.9 |
| Check | None | 21.10 | None | 23.3 |
| 113 | None | 21.80 | −0.66 | 22.1 |
| 113* | ..... | ..... | ..... | .... |
| 130 | None | 22.00 | −0.86 | 22.1 |
| 130* | ..... | ..... | ..... | .... |
| 131 | None | 22.10 | −0.96 | 22.4 |
| 131 | None | 22.00 | −0.86 | 22.1 |
| 132 | None | 20.86 | 0.28 | 21.5 |
| 132* | ..... | ..... | .... | .... |
| 133 | None | 21.60 | −0.46 | 23.5 |
| 133* | ..... | ..... | .... | .... |
| 413 | None | 21.08 | 0.06 | 23.0 |
| 413 | None | 22.05 | −0.91 | 23.8 |
| 414 | None | 20.98 | 0.16 | 22.4 |
| 414 | None | 21.40 | −0.26 | 22.7 |
| 415 | None | 21.62 | −0.48 | 22.4 |
| 415* | ..... | ..... | .... | .... |
| 416 | None | 21.30 | −0.16 | 22.1 |
| 416 | None | 21.08 | 0.06 | 21.5 |

* Culture contaminated.

the toxic limit of all of the organisms tested, as the solutions did not become turbid, and no growth was apparent. There were no changes in the nitrogen compounds present.

The nitrogen changes in a solution containing dl-alanine are shown in table 7. There was a much higher production of ammonia and a higher utilization of amino acid nitrogen in the solutions inoculated with the type A strain 130 and with organism 133 than in the solutions containing the type B organisms. Organism 113 was intermediate in action.

There was no change in the nitrogen present in the solutions inoculated with

the type A strains of *Rhizobium japonicum*, the organisms apparently being destroyed by the high concentration of alanine. The type B strains, however, apparently made some growth and utilized some of the amino acid nitrogen. Only a small amount of ammonia was produced by the type B strains. Nitrates and nitrites were absent from all of the solutions. The slight variation in the total nitrogen content of the solutions was probably within the limit of experimental error.

TABLE 7

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of dl-alanine to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | AMINO ACID TOTAL | AMINO ACID UTILIZED | TOTAL NITROGEN |
|---|---|---|---|---|
| Check | 0.56 | 16.38 | None | 17.42 |
| Check | 0.56 | 16.58 | None | 17.42 |
| 113 | 1.96 | 13.26 | 1.82 | 16.80 |
| 113 | 2.80 | 13.12 | 1.12 | 17.12 |
| 130 | 3.92 | 9.28 | 3.84 | 16.96 |
| 130 | 4.76 | 8.44 | 3.84 | 17.42 |
| 131 | 1.12 | 14.84 | 1.08 | 17.26 |
| 131 | 1.12 | 15.28 | 0.64 | 18.20 |
| 132 | 0.56 | 16.40 | 0.08 | 17.92 |
| 132 | 0.56 | 16.96 | −0.48 | 16.80 |
| 133 | 3.92 | 9.60 | 3.52 | 17.12 |
| 133 | 2.80 | 10.94 | 3.30 | 17.92 |
| 413 | 0.28 | 17.10 | −0.62 | 16.80 |
| 413 | 0.28 | 16.92 | −0.44 | 17.42 |
| 414 | 0.84 | 13.34 | 2.86 | 16.68 |
| 414 | 0.84 | 14.28 | 1.92 | 16.80 |
| 415 | 0.28 | 15.06 | 1.42 | 16.80 |
| 415 | 1.40 | 13.82 | 1.82 | 17.92 |
| 416 | 0.28 | 16.36 | 0.12 | 17.63 |
| 416 | 0.28 | 16.88 | −0.40 | 18.20 |

Table 8 shows the nitrogen changes produced in dl-alpha-amino-n-butyric acid. The production of ammonia and the utilization of the amino acid nitrogen were again much greater in the solutions inoculated with the alfalfa organisms 130 and 133. There was a very small amount of ammonia produced by the type B alfalfa organisms and a slightly larger amino acid utilization. The soybean bacteria were apparently not able to utilize this amino acid although one result does seem to show a slight utilization. The cultures were all free from nitrates. Traces of nitrites were present in the check solutions but no nitrites could be detected in any of the inoculated solutions. The variations in the total nitrogen content were too small to be of significance.

The changes in dl-valine are shown in table 9. The results obtained with both species of Rhizobium were variable and did not show any appreciable util-

TABLE 8

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of dl-amino-n-butyric acid to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | AMINO ACID TOTAL | AMINO ACID UTILIZED | TOTAL NITROGEN |
|---|---|---|---|---|
| Check | None | 14.68 | None | 14.85 |
| Check | None | 14.68 | None | 15.12 |
| 113 | 0.56 | 6.76 | 7.36 | 15.40 |
| 113 | 0.56 | 13.58 | 0.54 | 15.12 |
| 130 | 1.12 | 11.48 | 2.08 | 15.68 |
| 130* | .... | ..... | .... | ..... |
| 131 | 0.56 | 13.21 | 0.91 | 15.68 |
| 131* | .... | ..... | .... | ..... |
| 132 | 0.28 | 13.20 | 1.20 | 14.57 |
| 132 | 0.28 | 13.25 | 1.15 | 14.85 |
| 133 | 2.80 | 8.23 | 3.65 | 15.12 |
| 133 | 3.08 | 7.79 | 3.81 | 16.53 |
| 413 | None | 14.54 | 0.14 | 15.68 |
| 413* | ..... | ..... | .... | ..... |
| 414 | None | 14.65 | 0.03 | 14.57 |
| 414 | 0.28 | 13.67 | 0.73 | 15.12 |
| 415 | None | 14.80 | 0.12 | 15.96 |
| 415* | ..... | ..... | .... | ..... |
| 416 | None | 14.65 | 0.03 | 14.28 |
| 416* | ..... | ..... | .... | ..... |

* Culture contaminated.

TABLE 9

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of dl-valine to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | AMINO ACID TOTAL | AMINO ACID UTILIZED | TOTAL NITROGEN |
|---|---|---|---|---|
| Check | 0.28 | 13.31 | None | 12.60 |
| Check | 0.28 | 13.42 | None | 13.16 |
| 113 | 0.28 | 13.57 | −0.21 | 12.05 |
| 113* | .... | ..... | .... | ..... |
| 130 | 0.56 | 12.82 | 0.26 | 12.60 |
| 130 | 0.56 | 12.86 | 0.22 | 13.16 |
| 131 | 0.28 | 13.57 | −0.21 | 13.44 |
| 131* | .... | ..... | .... | ..... |
| 132 | 0.28 | 13.00 | 0.36 | 13.72 |
| 132 | 0.28 | 12.96 | 0.40 | 13.44 |
| 133 | 0.56 | 12.99 | 0.09 | 14.56 |
| 133 | 0.28 | 13.17 | 0.19 | Lost |
| 413 | 0.28 | 13.57 | −0.21 | 14.56 |
| 413* | .... | ..... | .... | ..... |
| 414 | 0.28 | 13.57 | −0.21 | 13.72 |
| 414 | 0.28 | 13.68 | −0.32 | 13.16 |
| 415 | 0.28 | 13.31 | 0.05 | 13.44 |
| 415 | 0.28 | 13.21 | 0.15 | 14.56 |
| 416 | 0.28 | 13.14 | 0.22 | 14.28 |
| 416* | .... | ..... | ..... | ..... |

* Culture contaminated.

ization of amino acid nitrogen nor any production of ammonia. The slight increase in total nitrogen in many of the inoculated solutions was not significant. There were no nitrates nor nitrites present in any of the solutions.

The nitrogen changes brought about in a solution containing d-glutamic acid are shown in table 10. Although there was no ammonia present in any of the cultures after the 5-week incubation period there was an appreciable utilization of the amino acid nitrogen in all of the tests. This was small in the solutions inoculated with *Rhizobium meliloti*, and there was no difference between the various strains of organisms in this species. The utilization was slightly

TABLE 10

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of d-glutamic acid to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | AMINO ACID TOTAL | AMINO ACID UTILIZED | TOTAL NITROGEN |
|---|---|---|---|---|
| Check | None | 10.42 | None | 10.08 |
| Check | None | 10.14 | None | 10.08 |
| 113 | None | 9.44 | 0.84 | 11.48 |
| 113 | None | 9.82 | 0.46 | 10.08 |
| 130 | None | 9.71 | 0.57 | 10.63 |
| 130* | ..... | .... | .... | ..... |
| 131 | None | 9.46 | 0.82 | 10.92 |
| 131 | None | 9.36 | 0.92 | 10.08 |
| 132 | None | 9.19 | 1.09 | 10.63 |
| 132 | None | 9.19 | 1.09 | 10.15 |
| 133 | None | 9.41 | 0.87 | 11.54 |
| 133 | None | 9.25 | 1.03 | 10.50 |
| 413 | None | 8.77 | 1.51 | 10.08 |
| 413 | None | 8.56 | 1.72 | 10.63 |
| 414 | None | 0.26 | 10.02 | 10.63 |
| 414 | None | Lost | Lost | 10.36 |
| 415 | None | 0.53 | 9.75 | 12.88 |
| 415 | None | 0.69 | 9.59 | 10.92 |
| 416 | None | 8.82 | 1.46 | 9.52 |
| 416* | ..... | .... | .... | ..... |

* Culture contaminated.

greater by the type A strains of *Rhizobium japonicum*, and very much greater by organisms 414 and 415, which are type B strains. These latter had utilized nearly all of the amino acid nitrogen. Qualitative tests showed small amounts of nitrites in the check solutions, but none in any of the inoculated solutions, indicating a utilization of the small amount present. Nitrates were not found in any of the solutions. The total nitrogen determinations showed no appreciable gain or loss in the nitrogen content during the incubation period.

Table 11 shows the nitrogen changes produced in l-cystine solution. In order to provide a solution of cystine having the desired concentration it was

necessary to add hydrochloric acid to the medium, thus producing a salt which was readily soluble. Sufficient sodium hydroxide was then added to bring the reaction up to a pH of 7.0. There was apparently a small amount of ammonia in the reagents which was utilized by most of the organisms. Although the Van Slyke determination did not measure all of the amino acid nitrogen it was apparent that some amino nitrogen was utilized by all of the cultures tested, the amounts varying more widely within the species than between the two species tested. Of the solutions inoculated with *Rhizobium meliloti* the one in which culture 130 (the type A strain) was growing showed a greater utilization

TABLE 11

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of l-cystine to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | AMINO ACID TOTAL | AMINO ACID UTILIZED | TOTAL NITROGEN |
|---|---|---|---|---|
| Check | 0.56 | 6.88 | None | 9.52 |
| Check | 0.84 | 6.54 | None | 11.21 |
| 113 | None | 6.06 | 0.65 | 10.37 |
| 113 | None | 5.94 | 0.77 | 10.37 |
| 130 | None | 3.64 | 3.07 | 12.32 |
| 130* | ..... | .... | .... | ..... |
| 131 | None | 5.77 | 0.94 | 10.64 |
| 131 | 0.56 | 5.27 | 1.44 | 9.52 |
| 132 | None | 5.93 | 0.78 | 11.77 |
| 132 | None | 6.04 | 0.67 | 11.21 |
| 133 | None | 6.12 | 0.59 | 11.21 |
| 133 | None | 6.12 | 0.59 | 11.77 |
| 413 | None | 5.37 | 1.34 | 11.64 |
| 413 | None | 5.26 | 1.45 | 8.41 |
| 414 | 0.56 | 3.62 | 3.09 | 11.49 |
| 414 | 0.28 | 3.86 | 2.85 | 10.09 |
| 415 | None | 4.57 | 2.14 | 8.69 |
| 415 | None | 4.67 | 2.04 | 9.25 |
| 416 | None | 5.16 | 1.55 | Lost |
| 416 | None | 5.74 | 0.97 | 10.09 |

* Culture contaminated.

of amino acid nitrogen than those containing the type B strains. Organisms 113 and 133 utilized about the same amount of amino nitrogen as the type B strains.

In the solutions inoculated with *Rhizobium japonicum* the reverse was true, the type B strains utilizing more than the type A strains, but the differences between these two were not large. The differences in the total nitrogen content were of little significance. Nitrates were not present in any of the solutions. Traces of nitrites were found only in the check solutions, the small amount present evidently being utilized in the inoculated solutions.

The nitrogen changes in l-tyrosine solution are given in table 12. There was no ammonia present in any of the solutions at the end of the 5-week incubation period. The amino nitrogen utilized varied from 0.60 mgm. in culture 113 to 2.73 mgm. in culture 416. The color of the solutions at the end of the incubation period were found to be much the same as in the previous test with tyrosine, except that the two strains of soybean bacteria 414 and 415 did not cause any change in the color of the medium. The amount of growth seemed to be much greater in the solutions inoculated with 414 and 415 than in any of the others, but the utilization of amino acid nitrogen was less than in the other

TABLE 12

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of l-tyrosine to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | AMINO ACID TOTAL | AMINO ACID UTILIZED | TOTAL NITROGEN |
|---|---|---|---|---|
| Check | None | 8.17 | None | 9.10 |
| Check | None | 8.69 | None | 10.08 |
| 113 | None | 7.83 | 0.60 | 7.74 |
| 113 | None | 7.40 | 1.03 | 8.68 |
| 130 | None | 7.20 | 1.23 | 8.40 |
| 130 | None | 7.42 | 1.01 | 8.96 |
| 131 | None | 6.22 | 2.21 | 8.96 |
| 131 | None | 6.01 | 2.42 | 8.68 |
| 132 | None | 6.05 | 2.38 | 9.52 |
| 132 | None | 5.84 | 2.59 | 10.08 |
| 133 | None | 6.86 | 1.57 | 8.40 |
| 133 | None | 7.10 | 1.33 | 8.96 |
| 413 | None | 5.82 | 2.61 | 8.68 |
| 413 | None | 5.88 | 2.55 | 7.74 |
| 414 | None | 6.31 | 2.12 | 7.74 |
| 414 | None | 6.58 | 1.85 | 7.56 |
| 415 | None | 6.90 | 1.53 | 7.56 |
| 415 | None | 6.94 | 1.49 | 8.96 |
| 416 | None | 5.79 | 2.64 | 7.28 |
| 416 | None | 5.70 | 2.73 | 7.28 |

strains of soybean organisms, and only slightly higher than in the solutions inoculated with the type A alfalfa organisms. Apparently the production of color was not dependent upon the utilization of amino acid nitrogen, but rather was the result of enzyme action. There were no nitrates present in any of the solutions, and the differences in the total nitrogen content, although usually showing a slight loss in nitrogen present, were not of any great importance. Nitrites were present in small amounts in the check solutions but had entirely disappeared from the inoculated media.

The changes produced in dl-phenylalanine are shown in table 13. The amounts of amino acid nitrogen utilized varied from —0.03 mgm. to 1.06 mgm.

TABLE 13

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of dl-phenylalanine to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | AMINO ACID TOTAL | AMINO ACID UTILIZED | TOTAL NITROGEN |
|---|---|---|---|---|
| Check | None | 9.25 | None | 9.96 |
| Check | None | 9.30 | None | 9.96 |
| 113 | None | 8.92 | 0.35 | 9.96 |
| 113 | None | 9.25 | 0.02 | 9.80 |
| 130 | None | 8.98 | 0.29 | 10.90 |
| 130 | None | 8.98 | 0.29 | 9.96 |
| 131 | None | 9.14 | 0.13 | 10.27 |
| 131 | None | 9.30 | −0.03 | 10.58 |
| 132 | None | 9.25 | 0.02 | 9.34 |
| 132* | ..... | .... | ..... | .... |
| 133 | None | 8.21 | 1.06 | 9.80 |
| 133 | None | 8.85 | 0.42 | 9.50 |
| 413 | None | 9.28 | −0.01 | 9.65 |
| 413 | None | 8.64 | 0.63 | 9.96 |
| 414 | None | 8.48 | 0.79 | 9.25 |
| 414* | ..... | .... | ..... | .... |
| 415 | None | 9.30 | −0.03 | 9.65 |
| 415 | None | 9.20 | 0.07 | 10.27 |
| 416 | None | 9.02 | 0.25 | 9.25 |
| 416 | None | 8.87 | 0.40 | 10.12 |

* Culture contaminated.

TABLE 14

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of p-amino benzoic acid to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | AMINO ACID TOTAL | TOTAL NITROGEN |
|---|---|---|---|
| Check | None | | 10.36 |
| Check | None | | 10.08 |
| 113 | None | | 10.08 |
| 113 | None | | 10.36 |
| 130 | None | | 10.92 |
| 130 | None | | 10.08 |
| 131 | None | | 9.80 |
| 131 | None | | 9.24 |
| 132 | None | | 10.36 |
| 132 | None | Not determined | 10.08 |
| 133 | None | | 10.64 |
| 133 | None | | 9.52 |
| 413 | None | | 10.64 |
| 413 | None | | 9.80 |
| 414 | None | | 9.52 |
| 414 | None | | 9.52 |
| 415 | None | | 8.96 |
| 415 | None | | 9.52 |
| 416 | None | | 9.52 |
| 416 | None | | 9.80 |

There did not seem to be any consistant relationship between the organism and the amount of amino acid nitrogen utilized. No ammonia, nitrates, or nitrites were found in any of the solutions. The variations in the total nitrogen content were within the experimental error.

The results obtained with p-amino benzoic acid are shown in table 14. Neither *Rhizobium meliloti* nor *Rhizobium japonicum* was able to produce any ammonia from this amino acid. The determination of the amount of amino nitrogen could not be made by the Van Slyke method as the amino group in positions other than on the alpha carbon atom reacts very slowly. No nitrates

TABLE 15

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of asparagine to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | TOTAL NITROGEN |
|---|---|---|
| Check | 0.56 | 23.50 |
| Check | 0.56 | 23.50 |
| 113 | 3.92 | 24.06 |
| 113 | 3.92 | 23.24 |
| 130 | 4.48 | 23.50 |
| 130 | 4.20 | 24.06 |
| 131 | 2.80 | 23.80 |
| 131 | 4.76 | 23.50 |
| 132 | 4.48 | 24.06 |
| 132 | 4.48 | 23.24 |
| 133 | 3.36 | 23.80 |
| 133 | 2.80 | 23.80 |
| 413 | 1.96 | 24.50 |
| 413 | 2.52 | 22.96 |
| 414 | 10.92 | 18.75 |
| 414 | 10.36 | 19.22 |
| 415 | 10.36 | 18.48 |
| 415 | 5.60 | 17.92 |
| 416 | 3.36 | 24.64 |
| 416 | 2.80 | 24.06 |

nor nitrites were present. The variations in the total nitrogen content of the solutions, although seeming to indicate the loss of small amounts of nitrogen from the inoculated solutions, were small. The four solutions containing the type B strains of soybean bacteria (414 and 415) showed a dark brown to black color at the end of the incubation period. This was even darker than the color produced in tyrosine by the type A strains of *Rhizobium japonicum.*

Table 15 shows the changes in asparagine caused by *Rhizobium meliloti* and *Rhizobium japonicum.* There was a marked production of ammonia by all of the organisms tested, the greatest production occurring in the solutions inoculated with the type B strains of soybean organisms. The results of the amino

acid determinations were not included in the tables because the tests were made before the removal of the ammonia, and include a part of it. They did show in all cases a slight increase in the amount determined as a result of inoculation, indicating that some of the ammonia measured must have been produced from the amido group. There was little difference in the production of ammonia by the alfalfa bacteria tested or by the type A strains of soybean bacteria. However, the amount of ammonia produced by the type B strains of *Rhizobium japonicum* was much greater than by any of the other organisms tested. The loss of ammonia probably was the cause of the decrease in the total nitrogen

TABLE 16

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of urea to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | AMINO ACID TOTAL | TOTAL NITROGEN |
|---|---|---|---|
| Check | 4.20 | | 51.8 |
| Check | 3.92 | | 50.9 |
| 113 | 16.23 | | 22.6 |
| 113 | 16.80 | | 21.0 |
| 130 | 17.08 | | 24.6 |
| 130 | 18.20 | | 22.1 |
| 131 | 13.17 | | 21.0 |
| 131 | 15.12 | | 26.8 |
| 132 | 7.78 | | 22.7 |
| 132* | .... | Not determined | .... |
| 133 | 6.44 | | 21.8 |
| 133 | 6.44 | | 30.0 |
| 413 | 6.16 | | 42.0 |
| 413 | 5.88 | | 42.5 |
| 414 | 4.76 | | 41.7 |
| 414 | 4.76 | | 41.7 |
| 415 | 5.03 | | 35.8 |
| 415 | Lost | | 35.2 |
| 416 | 4.76 | | 38.4 |
| 416 | 4.48 | | 40.3 |

* Culture contaminated.

content in the solutions inoculated with cultures 414 and 415. Nitrates and nitrites were absent from all of the solutions.

The nitrogen changes in a solution containing urea are shown in table 16. The production of ammonia, although significant in all of the tests, was much greater in the solutions inoculated with strains 113, 130, and 131 of *Rhizobium meliloti*. Amino nitrogen was not determined. There was a significant loss of nitrogen in all of the inoculated solutions, probably due to a loss of ammonia. This loss was much greater in the solutions inoculated with the alfalfa bacteria.

Table 17 shows the changes produced in a solution containing ammonium sulfate. A small amount of ammonia was utilized by all of the organisms, the amount varying considerably. Usually the amount utilized was small, often less than the amount of nitrogen utilized by the same organisms grown in amino acid solutions. There was no significant difference in the utilization by the two species nor by the different types within the species. The determination of total nitrogen did not show any marked change in the nitrogen content of the inoculated solutions. Nitrates and nitrites were not found in any of the solutions.

TABLE 17

*Nitrogen changes produced by Rhizobium meliloti and Rhizobium japonicum in a solution containing 1.0 gm. of ammonium sulfate to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA | AMMONIA UTILIZED | TOTAL NITROGEN |
|---|---|---|---|
| Check | 21.20 | None | 22.02 |
| Check | 21.20 | None | 21.32 |
| 113 | 20.17 | 1.03 | 21.82 |
| 113 | 5.60 | 15.60 | 21.26 |
| 130 | 19.22 | 1.98 | 22.65 |
| 130 | 19.60 | 1.60 | 22.65 |
| 131 | 17.36 | 3.84 | 23.40 |
| 131 | 14.28 | 6.92 | 22.65 |
| 132 | 19.22 | 1.98 | 21.56 |
| 132 | 19.22 | 1.98 | 21.82 |
| 133 | 19.22 | 1.98 | 21.82 |
| 133* | ..... | .... | ..... |
| 413 | 19.88 | 1.32 | 21.26 |
| 413* | ..... | .... | ..... |
| 414 | 16.80 | 4.40 | 20.22 |
| 414 | 17.36 | 3.84 | 20.22 |
| 415 | 18.20 | 3.00 | 22.40 |
| 415 | 18.20 | 3.00 | 21.26 |
| 416 | 15.68 | 5.52 | 21.00 |
| 416 | 12.60 | 8.60 | 21.26 |

* Culture contaminated.

The changes in the form of nitrogen in solutions containing potassium nitrate are shown in table 18. The amounts of amino acid and ammonia were small in all of the solutions and it is probable that all of the nitrogen was present in the amino form, which was produced during the utilization of the nitrates. The decrease in the amount of nitrates was partly the result of the utilization of this form of nitrogen by the bacteria. Qualitative tests for nitrites showed that they were absent from the two check solutions, but the test was very strong in all of the inoculated solutions. This leads to the assumption that the nitrites were produced by the bacteria, either as the first step in the utilization of

the nitrates, or as a by-product. On account of lack of solution it was not possible to make total nitrogen determinations in all of the solutions, but equal portions of the duplicate solutions were used. The frequent contamination met with lowered the number of determinations still more, but the results showed a loss of nitrogen from the inoculated flasks.

TABLE 18

*Nitrogen changes produced by Rhizobium melioloti and Rhizobium japonicum in a solution containing 1.0 gm. of potassium nitrate to a liter*

(Results expressed as milligram nitrogen to 100 cc. of solution)

| ORGANISM | AMMONIA AND AMINO ACID | NITRATES AND NITRITES | | TOTAL NITROGEN |
|---|---|---|---|---|
| | | Total | Utilized | |
| Check | 0.32 | 16.20 | None | 15.12 |
| Check | 0.38 | 15.08 | None | ..... |
| 113 | 0.32 | 12.27 | 3.37 | 14.28 |
| 113 | 1.89 | 14.80 | 0.84 | ..... |
| 130 | 0.75 | 9.19 | 6.45 | ..... |
| 130* | .... | ..... | .... | ..... |
| 131 | 0.32 | 10.31 | 5.33 | 11.47 |
| 131 | 0.22 | 10.88 | 4.76 | ..... |
| 132 | 0.43 | 5.28 | 10.36 | 11.47 |
| 132 | 0.27 | 9.75 | 5.89 | ..... |
| 133 | 1.94 | 8.91 | 6.73 | ..... |
| 133* | .... | ..... | .... | ..... |
| 413* | .... | ..... | .... | ..... |
| 413* | .... | ..... | .... | ..... |
| 414 | 0.92 | 6.95 | 8.69 | ..... |
| 414* | .... | ..... | .... | ..... |
| 415 | 0.49 | 14.23 | 1.41 | ..... |
| 415* | .... | ..... | .... | ..... |
| 416* | .... | ..... | .... | ..... |
| 416* | .... | ..... | .... | ..... |

* Culture contaminated.

## DISCUSSION

A general study of the data presented shows that the two species of legume bacteria tested were able to utilize some of the nitrogen present in a number of organic and inorganic compounds. During this utilization ammonia was produced from certain of the amino acids in excess of that utilized by the bacteria. The reaction probably was the result of the action of a group of enzymes known as deaminases which activate the hydrolysis of the amino acid, producing ammonia as one of the end products. Whether this preceded the utilization of the nitrogen or whether it occurred later was not definitely proved, although two facts indicate that the latter hypothesis may be the correct one.

In the first place no ammonia was found in the inoculated solutions containing valine, glutamic acid, tyrosine, cystine, amino benzoic acid, or phenylala-

nine. The utilization of some of these compounds was so small that small amounts of ammonia might have been produced and all of this ammonia later utilized, leaving no measurable amount present at the end of the incubation period. However, more of the amino acid nitrogen was utilized from some of the above compounds and at least small amounts of ammonia would probably still be present, if it had been produced.

Secondly, the utilization of the amino nitrogen by the type B strains of *Rhizobium japonicum* growing in glutamic acid solution was so much greater than the utilization of the ammonia furnished by ammonium sulfate that it seems probable, at least in this instance, that the utilization was direct.

Both amino and amido nitrogen were apparently changed in the solutions containing asparagine, but the degree of utilization cannot be arrived at from the determinations which were made. Urea was probably hydrolyzed by the action of urease previous to utilization, for, although no tests were made to determine the actual form utilized, this change seems to have occurred readily in the inoculated solutions.

The nitrogen present in ammonium sulfate was utilized in varying amounts by all of the organisms tested. Whether this was first built up into amino acid and then synthesized into more complex compounds was not determined. The amounts used were usually larger in the solutions inoculated with *Rhizobium japonicum* than in those inoculated with *Rhizobium meliloti*. This may be explained by the fact that the soybean organisms tend to produce an alkaline reaction, which is more suitable than the acid reaction that would occur as a result of the sulfuric acid in the solution following the utilization of the ammonia.

Nitrates were also used in varying amounts by all of the organisms tested. The presence of nitrites in all of the cultures indicates that this may be the first product and that this is then used in the building up of the more complex nitrogenous compounds. The wide variation in the amino and ammonia nitrogen present in the solutions containing nitrates does not allow any conclusions to be dawn regarding their production. The loss of nitrogen may have been the result of a loss of free nitrogen produced during the reduction process which occurred.

Although no tests were started using a nitrite compound as the nitrogen carrier the frequent occurrence of nitrites in the check solutions and their absence in the inoculated solutions indicates that nitrites may be used, at least in small amounts by the legume bacteria.

Inasmuch as ammonia and nitrites have been found to be produced from other nitrogenous compounds by legume bacteria the presence of either of these in the nodules or in solutions which contain these bacteria does not indicate that they are the forms in which the nitrogen is fixed. On the other hand, the ability of the legume bacteria to produce ammonia from a number of compounds does point to the possibility that ammonia may be the product which the plant obtains from the nodule. No attempt was made to test for or obtain bacteroids in these experiments. If these are the forms which are re-

sponsible for fixation, as some writers suggest, it would appear that they are more active physiologically, and thus, it is possible that in this form the changes produced would be of much greater magnitude than those found.

In regard to the value of the tests made as a means of differentiating between species a few points may be emphasized.

There were apparently no differences in the changes produced by the two species in peptone, dl-valine, dl-phenylalanine, p-amino benzoic acid, ammonium sulfate, and potassium nitrate. In certain other compounds such as dl-alanine, l-tyrosine, d-glutamic acid, asparagine, and l-cystine the differences between the two types within the species were as great as the differences between species. However, there were observable differences between *Rhizobium meliloti* and *Rhizobium japonicum* in some of the solutions.

The changes produced in urea were consistently greater in the solutions inoculated with *Rhizobium meliloti* than in those inoculated with *Rhizobium japonicum*. This was very noticeable in the total nitrogen content, the cultures inoculated with alfalfa organisms having lost much more nitrogen than those inoculated with the soybean organisms. If this loss was the result of a loss of ammonia, then the production of ammonia must also have been much greater.

The experiments with glycocoll gave widely differing results. In the first test (table 1) there appeared to be a very significant difference in the ability of the two species studied to produce ammonia and utilize the nitrogen. In the second test, however, no utilization was apparent in any of the solutions. This observed difference may have been due to the increase in the amount of glycocoll added in the second test, making it toxic to both species, whereas the smaller amount used in the first test was toxic only to *Rhizobium japonicum*.

The colors produced in the tyrosine solutions were probably due to the difference in the enzyme action. The action of tyrosinase proceeds in three steps, the first substance produced being red, then this is converted to a colorless compound, which in turn is changed to melanin, a black compound. In acid solutions, tyrosinase changes the tyrosine only partially with the production of a compound having a red color, whereas in alkaline solutions the reaction proceeds farther with the production of melanin. These characteristics then appear to be linked with reaction rather than with the character of the enzyme produced, and can not be considered as being more accurate than measurements of reaction change. The lack of color production in the solutions inoculated with the type B organisms does not prove that the enzyme tyrosinase was absent, but its action may have been intermediate between the alfalfa and type A soybean organisms.

The results obtained with the solutions of dl-amino-n-butyric acid indicate that this amino acid may help in differentiating *Rhizobium meliloti* and *Rhizobium japonicum*. There was a significant production of ammonia and also a marked utilization of amino acid nitrogen in all of the solutions inoculated with the alfalfa bacteria, but only one culture of the soybean bacteria showed any production of ammonia. It is probable, in view of the other results, that this one culture had become contaminated.

By properly regulating the concentration of the amino acid added it appears that any one of the three simple mono-amino-mono-carboxylic acids (glycocoll, alanine, and amino-butyric acid) may aid in differentiating *Rhizobium meliloti* from *Rhizobium japonicum*. There may also be found to be a difference in the value of the dextro- and levo-amino acids which did not appear in the tests, which were, for the most part, conducted with the racemic mixtures of these two.

More striking differences were observed in the action of the types within the species. Type A strain of the alfalfa bacteria utilized much more of the amino acid nitrogen and produced more ammonia from dl-alanine than did the type B strains. Inasmuch as the test for ammonia can be made much more easily than the test for amino acids it may be possible to adapt this test to distinguish between these two types. Type A strains of soybean bacteria did not produce any ammonia nor use any of the amino nitrogen from dl-alanine, but the type B strains were able to make use of some of this nitrogen and to produce small quantities of ammonia. This difference could be made the basis of a test to differentiate these types.

The results obtained by inoculating *Rhizobium meliloti* into solutions containing dl-amino-n-butyric acid are similar to those obtained in alanine solutions. The type A strains apparently used more of the amino acid nitrogen and produced more ammonia than did the type B strains. None of the soybean bacteria tested were able to change appreciable amounts of dl-amino-n-butyric acid.

There were no significant differences in the utilization of d-glutamic acid by the alfalfa bacteria, but such differences were found in the solutions inoculated with *Rhizobium japonicum*. The type B strains of this latter species utilized nearly all of the amino acid nitrogen present, while the type A strains utilized only about one-sixth of the amino nitrogen. There was a considerable difference in the turbidity of the solutions, indicating more rapid growth by the type B strains, and it is quite probable that similar differences in growth would occur on agar slants containing glutamic acid.

Although there appeared to be a slight difference in the utilization of l-cystine by the two types of *Rhizobium meliloti* only one result was obtained for the type A strain, and no conclusions regarding the value of this amino acid in type differentiation can be drawn. There do, however, appear to be differences in the utilization by the different types of soybean organisms, the type B strains using about twice as much of the amino nitrogen as the type A strains.

The utilization of tyrosine was greater by the type B strains of *Rhizobium meliloti* than by the type A strains. The reverse was true in the solutions inoculated with *Rhizobium japonicum*, the type A strains using more of the tyrosine than the type B strains. The differences in color noted in the solutions inoculated with *Rhizobium japonicum* were probably due to a difference in the reaction of the solutions.

The value of p-amino-benzoic acid in the differentiating of types within species

appears not in the utilization of the amino nitrogen but in the production of the dark color in the solutions inoculated with the type B strains of *Rhizobium japonicum*. If this is found to be a constant characteristic of all of the type B strains but not of type A strains it will prove valuable in their separation. Asparagine also appears to have some merits which recommend its use in the differentiation of the two types of soybean bacteria. The production of ammonia was much greater in the solutions inoculated with the type B strains than in those containing the type A strains. Such a difference did not exist between the two types of alfalfa bacteria.

Dl-valine, dl-phenylalanine, urea, ammonium sulfate, and sodium nitrate did not appear to have any value in distinguishing the types in either *Rhizobium meliloti* or *Rhizobium japonicum*.

It is of interest to note that in the case of dl-alanine and l-cystine the utilization of the amino acid nitrogen was greater by the type A alfalfa strain and by the type B soybean strains, and in tyrosine the type B alfalfa strains and the type A soybean strains utilized more of the amino acid nitrogen. In no case was the greatest utilization found in the type A strains of both organisms or in the type B strains of the two. Apparently the type B strains of the alfalfa organisms are more closely related in their nitrogen utilization to the type A soybean strains, whereas the type A strains of alfalfa bacteria are more closely related to the type B soybean organisms.

One other fact deserves mention, namely, the high utilization of d-glutamic acid and asparagine by the type B strains of *Rhizobium japonicum*. These organisms are not as efficient in nitrogen fixation in the plant, and from the foregoing it appears as if this might be because these organisms were better able to utilize the nitrogen present in the plant, and were not forced to depend on the atmospheric nitrogen for their supply of this element.

It appears evident from the data presented here that the legume bacteria even when outside the plant may play some rôle in the nitrogen cycle. However, this is probably not as great as it would appear to be from the aforementioned experiments because of the lack of food material and the competition of other organisms. But they certainly can utilize soluble nitrogenous compounds and under certain conditions may be able to bring about a loss in the total nitrogen content of the medium upon which they are growing.

## SUMMARY AND CONCLUSIONS

Studies of the changes produced by *Rhizobium meliloti* and *Rhizobium japonicum* in nitrogenous compounds in solutions were made. The nitrogenous compounds tested were glycocoll, dl-alanine, dl-amino-n-butyric acid, dl-valine, d-glutamic acid, l-cystine, l-tyrosine, dl-phenylalanine, p-amino benzoic acid, urea, asparagine, peptone, ammonium sulfate, and potassium nitrate. Analyses were made for ammonia, nitrates, nitrites, amino acid nitrogen, and total nitrogen.

Changes were noted in the form of nitrogen present in the solutions contain-

ing glycocoll, dl-alanine, dl-amino-n-butyric acid, d-glutamic acid, l-cystine, l-tyrosine, asparagine, urea, ammonium sulfate, and potassium nitrate.

Ammonia was produced by *Rhizobium meliloti* from glycocoll, dl-alanine, dl-amino-n-butyric acid, asparagine, and urea. *Rhizobium japonicum* produced ammonia from dl-alanine, asparagine and urea.

Nitrates were utilized by both of the species tested. All of the inoculated solutions containing nitrates showed an increase in nitrites, which were probably produced during the utilization of the nitrates.

Nitrites were apparently utilized in small amounts by *Rhizobium meliloti* and *Rhizobium japonicum*.

Some of the nitrogen present in the amino group in glycocoll, dl-alanine, dl-amino-n-butyric acid, d-glutamic acid, l-cystine, and l-tyrosine was changed by *Rhizobium meliloti*. Changes in the amino nitrogen in dl-alanine, d-glutamic acid, l-cystine and l-tyrosine were produced by *Rhizobium japonicum*.

Differences in the changes produced by *Rhizobium meliloti* and *Rhizobium japonicum* were noted in glycocoll, l-tyrosine, dl-amino-n-butyric acid, and urea.

Differences were found in the action of the type A and the type B strains of *Rhizobium meliloti* in dl-alanine, dl-amino-n-butyric acid, and l-tyrosine. Differences also appeared in the changes produced by the type A and the type B strains of *Rhizobium japonicum* in dl-alanine, l-tyrosine, d-glutamic acid, l-cystine, p-amino benzoic acid, and asparagine.

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# A METHOD OF APPLICATION DESIGNED TO INSURE PROPER DISTRIBUTION OF FERTILIZERS IN FIELD TRIALS WITH FRUIT TREES

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A study of certain soils in the Sacramento Valley in which prune trees suffered a serious scorching of the foliage showed these soils to be distinctly low in both water-soluble and replaceable potassium. The division of plant nutrition in whose laboratories these analyses were made, also reported that these particular soils possessed a high fixing capacity for potassium and predicted that it would be difficult to increase the potassium content to any reasonable depth by the addition of potash salts to the surface of the soil.

Because of the low potash content of these soils and the fact that leaf scorch has been reported as associated with a deficiency of potash, the application of potassium salts in the prune orchards seemed a logical attack in an endeavor to remedy the condition.

A study of the root system of the prune trees in these soils revealed most of the visible roots to be in the second, third, and fourth feet of soil. Even though the trees were relatively shallow rooted the laboratory studies indicated that surface applications of ordinary commercial amounts could not be expected to influence the potash content in the major root zone.

Field studies confirmed the laboratory findings. The results in table 1 indicate that even when 50 pounds of sulfate of potash was broadcast on the surface of the soil over an area of approximately 200 square feet, no increase could be found in the second foot. The application was made in December and the soil samples were taken the following May. The surface 3 inches of soil which composed the dry mulch was discarded in sampling so that it would be more correct to say that no increase in either water-soluble or replaceable potash could be detected below the first 15 inches of soil. In any event the penetration of potash downward was not sufficient to reach many roots and a method had to be devised to overcome this difficulty. This was accomplished with the aid of a power spray rig such as is in use in almost any commercial orchard. The potash salts were dissolved in the spray tank. A specially constructed injection rod made up from ordinary pipe fittings was substituted for the spray rod or gun.

The rod was made of one-quarter-inch gas pipe which was threaded on both ends. The length of this rod depended upon the depth of injection desired. A

hole, one-eighth inch in diameter, was drilled through a plug, which in turn was screwed into a coupling and this in turn onto the one-quarter-inch gas pipe. The plug and coupling were then ground down to a point on an emery wheel. On the other end of the rod was fitted a one-quarter-inch globe valve which takes the standard spray hose nipple. A spade handle connected as indicated in plate 1, figure 1 affords a better grip than is permitted in the original construction. The plug and coupling together with the finished point are also shown.

Generally two such injection rods can be used with the average power spray rig. No difficulty is involved in forcing these into the ground as long as good pressure is maintained. The pressure gauge should register at least 200 pounds with both rods in action.

By the use of this apparatus it has been possible, in the soils mentioned, to increase the potash content to greater depths than a surface application would permit and to place the potash in the root zone. In the experimental field work it was possible to distribute 50 gallons of solution over the 200 square

TABLE 1

*Effect of method of application on penetration and distribution of potassium*[1]

| DEPTH OF SAMPLE | 50 POUNDS $K_2SO_4$ SURFACE | | NONE (CHECK) | | 50 POUNDS $K_2SO_4$ INJECTED | |
|---|---|---|---|---|---|---|
| | Water soluble K | Replaceable K | Water soluble K | Replaceable K | Water soluble K | Replaceable K |
| *feet* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* | *p.p.m.* |
| 0–1 | 9.7 | 520 | 0.9 | 114 | 4.2 | 246 |
| 1–2 | 2.1 | 79 | 1.1 | 70 | 3.2 | 197 |
| 2–3 | 1.9 | 41 | 1.3 | 44 | 13.8 | 592 |
| 3–4 | 2.8 | 45 | 1.1 | 38 | 3.4 | 163 |

feet at the approximate rate of one hole to every square foot to a depth of 3 feet. The time consumed in this operation with two men working was about 15 minutes.

Results of analyses of the soil from the plots which have been injected according to the method noted are shown in table 1. The effectiveness of this type of application in increasing the potash content in the lower depths is evident. The injection rods were generally inserted to a depth of 3 reet and this probably accounts for the greater increase observed at this depth. Much of the potash applied on the surface and unaccounted for in the analysis is undoubtedly fixed in the surface 3 inches which was discarded at the time of sampling. The method has no practical application but meets a particular requirement in experimental work in the field. It assures proper distribution of various salts throughout the soil mass where their effect is being studied on relatively deep-rooted deciduous fruit trees. The fixation of various cations through the

[1] The authors are indebted to P. A. Hibbard, associate chemist in the experiment station, for the analyses reported in table 1.

phenomenon of base exchange and the precipitation of phosphates may be such that surface application of fertilizers may never effect any chemical change in the mass of soil in which the major portion of the tree roots are found. Under these conditions the method of soil injection outlined may be of some assistance to those concerned with soil deficiency studies and fertilizer trials with fruit trees.

## PLATE 1

CONSTRUCTION AND OPERATION OF SOIL INJECTION ROD

FIG. 1. Parts used in the construction of the soil injection rod.
FIG. 2. Insuring proper distribution of fertilizer salts with soil injection rods.

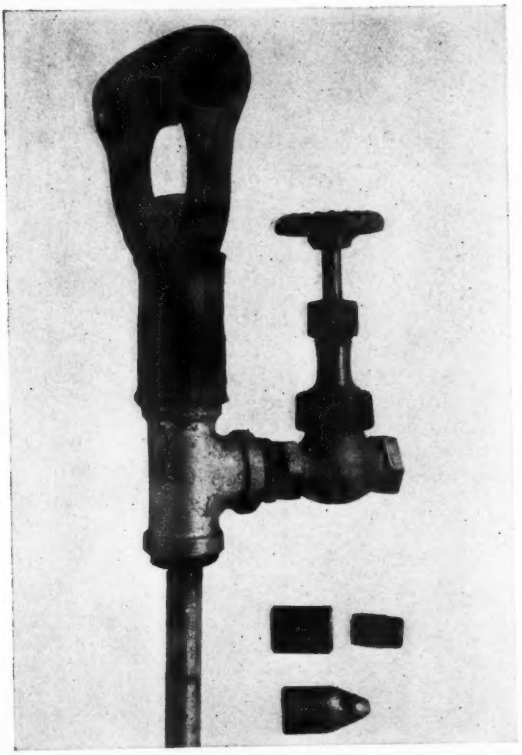

FIG. 1

FIG. 2